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THE USE OF AQUATIC VEGETATION AND INVERTEBRATES TO MONITOR CHLORINATED HYDROCARBONS IN THE LAKE HURON - LAKE ERIE CORRIDOR

R. A. C. PROJECT NO. 241 PL

Environment Ontario

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CHAPTER 1

SUMMARY, CONCLUSIONS & RECOMMENDATIONS

1. This report documents a study into the use of three aquatic biomonitors, namely the freshwater mussel *Elliptio complanata*, the mayfly nymph *Hexagenia limbata*, a benthic invertebrate species, and the aquatic macrophyte species *Myriophyllum spicatum*, as biomonitors of water quality in a quantitative sense. The main objective of this research was to investigate if these three biomonitors can provide quantitative information of ambient contaminant concentrations in Ontario waters and sediments. The reason for this research is that due to analytical difficulties with contaminant analysis in water, it is presently very difficult to monitor on a regular basis the water quality of rivers and lakes in Ontario on a quantitative level. The use of biomonitors may provide a method to quantitatively determine water quality in Ontario waters.

2. In chapter 3, a theoretical framework is developed for using biomonitors to measure quantitatively ambient contaminant concentrations. This theoretical framework is based on the mechanisms of chemical exchange between the organism and its environment. It is shown that when ambient concentrations are not fluctuating significantly over periods of 1 to 3 weeks, simple relationships exist between the concentration in the biomonitor and the concentration in the water or sediments. These biomonitor-water and biomonitor-sediment relationships thus provide a method to translate contaminant concentrations in the biomonitor to an ambient water or sediment

concentration.

The relationships between the concentration in the biomonitor and that in the ambient water or sediment are shown to depend on chemical, biological and environmental factors. To establish these relationships for a variety of chemicals and under varying biological and environmental conditions, a biomonitor needs to be calibrated. In essence, this calibration process is similar to the calibration of any other analytical instrument such as a pH-meter or a spectrophotometer.

3. A protocol is developed for the calibration of biomonitors: This protocol consists of a two step process. First, laboratory experiments are performed, in which the water-biomonitor and/or sediment-biomonitor concentration relationship are measured for different chemicals and under different conditions. Secondly, to ensure that the laboratory calibration applies under typical field conditions the water-biomonitor and/or sediment-biomonitor concentration relationships are tested in Ontario waters.

   This protocol has been applied to the calibration of the freshwater mussel *Elliptio complanata*, the benthic invertebrate *Hexagenia limbata*, and the aquatic macrophyte species *Myriophyllum spicatum*. The biomonitor calibration protocol outlined in this study pertains to conservative hydrophobic organic substances such as PCBs, chlorobenzenes, aromatic hydrocarbons and halogenated alkanes. Its applicability to metals is beyond the scope of this study.

4. Chapter 4 reports the calibration of the freshwater mussel *Elliptio complanata* for hexachlorobenzene (HCB) and

octachlorostyrene (OCS), both EMPPL substances. The laboratory study demonstrates that there exists a simple mussel-water concentration relationships for the two chemicals, namely

$C_w = 0.0000022.C_M/L_M$ for HCB

$C_w = 0.0000005.C_M/L_M$for OCS

where C_w is the concentration in the water (in ug/L), C_M is the concentration in the mussel (ug/kg wet weight) and L_M is the lipid content of the mussel. These relationships are valid when mussels are deployed for a period of 21 days (3 weeks) for HCB and 42 days (6 weeks) for OCS and when during the mussel deployment water concentrations do not vary more than by a factor 3.

The study further indicates that for mussel-water concentration relationships for other substances can be estimated from

$C_w = C_M/L_M.K_{ow}$

where K_{ow} is the chemical's 1-octanol-water partition coefficient.

5. Field validation of mussel-water concentration relationships in the Huron-Erie corridor appear to confirm the laboratory calibration studies. A rigorous laboratory-field comparison was not possible since no water concentration measurements were made. The field data indicate that chemical exchange between the water and the mussel is faster in the field than in the lab. This observation has no effect on the mussel-water

concentration relationships, but it indicates that mussel-water concentration ratios for HCB and OCS are reached at deployment times less than the 21 and 42 days observed in the laboratory tests.

6. It is recommended that in future research regarding the calibration of the freshwater mussel Elliptio complanata, laboratory and field calibration is extended to include a larger number of chemical substances. It is suggested that these chemicals vary in 1-octanol-water partition coefficient over several orders of magnitude.

   Of particular importance is the observation that contaminant uptake and elimination rates in the mussel may differ between laboratory and field deployments. This aspect needs to be further investigated, possibly by measuring contaminant kinetics in the mussel in the field. The field kinetics can then be compared with the laboratory kinetics.

   Since there are no documented protocols for measuring contaminant uptake, elimination and bioconcentration kinetics in mussels in the field, techniques and methodologies have to be developed for studying contaminant kinetics at the deployment sites.

7. Chapter 5 reports the calibration of the burrowing mayfly nymph *Hexagenia limbata*. Laboratory calibration was performed for hexachlorobenzene (HCB). It was found that the mayfly is interacting with both the sediments and the water. A sediment-water-mayfly concentration relationship was developed for HCB, i.e.

$C_s = 0.4.C_M - 800.C_w$

or

$C_w = 0.0005.C_M - 0.0013.C_s$

where C_w is the water concentration (ug/L), C_M is the mayfly concentration (ug/kg wet weight) and C_s is the sediment concentration (ug/kg dry weight). These relationships apply when mayflies are deployed for a period of at least 3 days and water and sediment levels do not vary more than a factor of 3.

8. Field calibration in Lake St. Clair for a series of PCBs and chlorobenzenes and OCS showed that mayfly/sediment concentration ratios varied from 0.14 for pentachlorobenzene to 0.71 for PCB-153, and were linearly correlated with the chemical's octanol-water partition coefficient when expressed on a logarithmic basis:

$\log (C_A/C_s) = 0.37\ [\pm\ 0.09].\log K_{ow} - 2.76\ [0.17]$

or

$C_A = 0.0017.K_{ow}^{0.37}.C_s$

where C_A is the mayfly concentration (ug/kg wet weight) and C_s is the sediment concentration (ug/kg dry weight).

9. A chemical equilibrium model of sediment-mayfly interactions, predicting a mayfly/sediment concentration of 0.5, was found

to give a satisfactory description of the field observations.

A dynamic sediment-water-mayfly model gives a more realistic description of sediment-water-mayfly interactions. However, when calibrated by data from the laboratory calibration, the model tends to overestimate the mayfly/sediment concentration ratios in Lake St. Clair by approximately a factor of 10. This indicates that the mayfly the laboratory calibration is inconsistent with the field observations. Similar findings have been documented by other researchers. The reason for this discrepancy is unclear and needs to be addressed.

10. Now that techniques have been developed for the laboratory calibration of the mayfly *Hexagenia limbata*, it is suggested that the laboratory calibration is extended to include a larger number of substances, such as PCBs, Chlorobenzenes and PAHs and others. By testing chemicals of varying octanol-water partition coefficient, it is possible to calibrate the mayfly for the chemical's hydrophobicity, which is the most important chemical factor controlling the water-organism exchange of contaminants. This calibration experiment would enable biomonitoring data of a greater number of chemicals to be interpreted in ambient water and sediment levels in a quantitative sense.

11. Chapter 6 reports the calibration of the aquatic macrophyte species *Myriophyllum spicatum*. In order to perform the calibration studies a plant analysis technique was developed. The laboratory calibration was conducted with a series of PCBs and chlorobenzenes, varying in log K_{ow} from 4 to 8.3. It was found that when deployed for a sufficiently long period of time, plants and water reach a chemical equilibrium. At

equilibrium, the concentration in the water can simply be determined from the concentration in the plant according to

$C_w = 180.C/K_{ow}$

where C_w is the water concentration (ug/L), C is the concentration in the plant (ug/kg wet weight) and K_{ow} is the chemical's octanol-water partition coefficient. This plant-water concentration relationship is applicable if the plants have been exposed to the water for a period of time t (days) that can be determined from

$t = 4.8 + 0.000045.K_{ow}$

where K_{ow} is the octanol-water partition coefficient of the chemical that is being monitored. In addition, for the plant-water concentration relationship to apply, water concentrations should not vary more than a factor of 3 during the deployment period.

12. Future research should address the field calibration.

13. This research has contributed to the development of simple protocols to interpret contaminant concentrations in biomonitors in terms of ambient contaminant concentrations in a quantitative sense.

It is concluded that all three biomonitors have the ability to monitor ambient contaminant levels in a quantitative sense. Now that techniques have been developed for plant tissue analysis, it is believed that the submerged aquatic macrophyte species Myriophyllum spicatum is the simplest biomonitor.

Chemical concentrations in the plant tissue can be relatively simply translated in a contaminant concentration in the water when the plants are deployed at locations where water concentrations are not greatly fluctuating such as at a reasonable distance from a point source or outfall. The other advantage of the plants as a biomonitor is that they do not have to be deployed. Plant collection from field locations and subsequent analysis may be the only activity involved on the part of monitoring agency. The disadvantage of the submerged aquatic plants over, for example, the mussel is that the plant has a 5 times lower chemical extraction power from the water than the mussel due to a lower lipid content. The collection of a larger number of plants can surmount this potential detection problem easily.

In various countries and over many years, the mussel has shown to be an excellent biomonitor of water quality. This study confirms this and stresses the possibility of the use of mussel data in a quantitative sense.

The mayfly has shown to be a useful biomonitor of contaminants in sediments. Our field study shows that sediment and mayfly concentrations are closely related. The applicability of the mayfly as a biomonitor may be associated with some practical difficulties, since the viability of the mayflies is limited to very specific sediment conditions. Consequently, the use of the mayfly as a biomonitor may also be limited to specific locations in Ontario such as Lake St. Clair.

14. In addition to the biomonitor calibration, this report also includes the findings of an investigation of contaminant levels at different trophic levels in the Huron-Erie corridor.

Of significance is the role that vegetation and wetlands may play in the regulation of contaminant transport and fate.

15. As part of this study two kinetic-chemical-transport models (Wastox and Toxiwasp) were calibrated for the Huron-Erie Corridor. These modelling studies have been reported independently.

16. In addition this research program supported:
    1) Four master theses
    2) eight scientific publications
    3) The development of a major analytical facility in South-western Ontario.

CHAPTER 2

INTRODUCTION

Of the estimated 40,000 chemicals found in the Great Lakes that are known to be of commercial and industrial value, less than 2% have been tested for effects on human health and the environment. It is generally accepted that MOE cannot wait until all chemicals are tested before implementing remedial actions, and thus there is a need for monitoring programs to determine not only changes in levels of contaminants, but also to provide information as to the hazard of these chemicals via exposure assessments.

Perhaps the most promising tool in exposure assessments is the development of biomonitors. Biomonitors were first deployed because of their ability to concentrate chemicals from the water to detectable levels using standard analytical techniques. An additional advantage was that chemical levels in organisms were thought to represent an integration of 'bioavailable' chemical levels over defined periods of time. With the appropriate calibration of these organisms, one can estimate an "average" water concentration of bioavailable chemical.

The aim of the present research was to calibrate both vegetative and invertebrate biomonitors in the laboratory and in the field. By determining how long it would take for chemicals to reach steady-state in the biomonitor, well defined biomonitoring protocols could be developed for a variety of chemicals. Research in both the field and laboratory components was focused on the freshwater mussel (*Elliptio complanata*), the mayfly (*Hexagenia limbata*) and on aquatic plant species (*Myriophyllum spicatum*).

For all species, a general model describing chemical uptake and elimination was used. The model is fully described in Chapter 3, along with a further development of theoretical considerations concerning the calibration of biomonitors.

Perhaps more important in a pragmatic sense, however, is the verification of toxicokinetic models predicting the time required for specific biomonitors to be exposed in order for a chemical to reach a steady-state in the biomonitor in its particular environment. With these models, the MOE will now be able to make reliable estimates of required exposure periods for organisms such as *Elliptio complanata, Hexagenia limbata, and Myriophyllum spicatum.*

Field work served as a verification of the laboratory toxicokinetic studies under conditions of low and high exposure, and as a means to test the sensitivity of the selected biomonitors to surrounding environments. This report is written in separate sections on the specific biomonitors. This format will allow the reader to have access to both the theoretical models used and the initial calibration of the selected biomonitors.

CHAPTER 3

BIOMONITORING: A QUANTITATIVE APPROACH

Introduction

One of the issues the MOE needs to address is the interpretation of levels of toxic organic chemicals in terms of exposure and effects to aquatic life. This can be achieved by direct measurement of quantities of trace organic chemicals in water and/or sediment. However, this is not only difficult because of the low concentration of most chemicals in the water, but it is often insufficient to determine either exposure or effects to aquatic organisms such as fish and benthos.

Since many organic chemicals tend to accumulate in aquatic organisms, resulting in concentrations in the organism exceeding those in the water by orders of magnitude, aquatic organisms can be used to monitor chemical concentrations in the water and to determine chemical exposure to aquatic life. The chemical concentration in the biomonitor can then be viewed as (i) a measure of the chemical concentration in the water or sediment and (ii) the exposure of the organism to the chemical, i.e. the "dose", which could be associated with a certain effect.

In order to relate observed body burdens of biomonitors to environmental chemical concentrations in the water, the relationship between the chemical concentrations in the water and the organism needs to be established. This relationship reflects the organism's capability to absorb chemical from the water, but also from food and other sources and the organism's potential to

depurate the chemical. This section will outline the approach that we follow to derive biomonitor specific relationships, which allow a quantitative assessment of environmental concentration in water and sediments from observed body burden in biomonitors. We will demonstrate chemical dependent and organism specific relationships relating chemical concentrations in aquatic organisms to chemical concentrations in the water and/or sediment, and show how these relationships can be used to set deployment strategies for biomonitors and to interpret biomonitoring data. We will also outline the limitations of this approach.

Uptake, Depuration and Bioaccumulation in Aquatic Biomonitors

In general, the expression describing simultaneous uptake of a chemical from food and water in aquatic organisms as well as the depuration of that chemical to the water (e.g. via the gills), into "faecal" matter and by metabolic transformation can be expressed as:

$$d(V_F.C_F)/dt = k_1.V_F.C_W - k_2.V_F.C_F + k_A.V_F.C_A - k_E.V_F.C_F - k_R.V_F.C_F \quad (1)$$

where C is concentration (mol/m^3), t is time (h), and the subscripts W refer to water, A to food, E to faeces, and F to the whole organism (Gobas et al. 1988, 1989). The organism is defined as the whole organism excluding the gill compartment, when present, and the gastro-intestinal (GI) tract. k_1, k_2, k_A and k_E are respectively the rate constants (h^{-1}) of chemical uptake from the water, elimination via the gills to the water, uptake from food, and elimination by egestion in the faeces. k_R is the rate constant (h^{-1}) for metabolic transformation of the chemical in the organism. Fish growth is included in the model through the term V_F, representing the volume of the organism in m^3. In this fashion

various growth scenarios can be treated by the model.

For many aquatic organisms, including fish, various mussel species, submerged aquatic macrophyte species and various benthic invertebrate species, equation 1 provides a useful framework to describe the exchange of chemicals between the organism/biomonitor and the organism's environment. The actual values of the rate constants, however, may vary from organism to organism. The rate constants k_1, k_2, k_E and k_R are therefore characteristic for the organism. They are also dependent on the properties of the chemical and environmental conditions. In other words, for every combination of chemical, organism and environmental condition, there is a unique set of rate constants that characterize the uptake, bioaccumulation and depuration of a chemical in an organism. To translate chemical concentrations in organisms or biomonitors to chemical concentrations in the ambient environment, e.g. the water, it is important to determine the appropriate set of kinetic rate constants. It is the purpose of this research project, to determine the rate constants for chemical uptake and elimination in several aquatic organisms which are presently used or considered for use as biomonitors by the OME. After these rate constants have been measured under different conditions and recipes have been developed that can be used to estimate the rate constants under different conditions, the biomonitor is in essence calibrated and chemical concentrations in the biomonitor can be related to ambient concentrations in a quantitative sense.

The Kinetic and the Fugacity Approach

Equation 1 can also be expressed by fugacity expressions (Gobas et al. 1988):

$$V_F . Z_F . df_F/dt = D_F . (f_w - f_F) + D_A . f_A - D_E . f_F - D_R . f_F \quad (2)$$

where V is volume (m^3), Z is the chemical's fugacity capacity ($mol/m^3.Pa$) in a phase, and f is the chemical's fugacity (Pa). D_F is the net transport parameter (mol/Pa.h) for chemical transfer between water and fish across the respiratory surface (e.g. gills). D_A is the transport parameter for chemical uptake from food into the organism across the gastro-intestinal (GI)-tract. The transport parameter D_E (mol/Pa.h) describes chemical elimination in the faeces. D_R (mol/Pa.h) is the transformation parameter for metabolic transformation of chemical in the organism. The transport parameters D_F, D_A, and D_E include all transport processes involved in solute transfer between the water, food, and faeces, respectively, and the solute's final storage site in the fish.

Integration of equation 1, assuming a constant C_w and C_A, an initial C_F of zero and a constant fish volume with time, gives:

$$C_F = \{C_w . [k_1/(k_2+k_E+k_R)] + C_A . [k_A/(k_2+k_E+k_R)]\} . \{1-\exp-(k_2+k_E+k_R) . t\} \quad (3)$$

This equation describes the chemical concentration in the organism with time when an uncontaminated organism, with no initial chemical concentration, is exposed to water with a constant concentration of the chemical for a certain length of time.

Since C_F is f_F/Z_F, C_w is f_w/Z_w and C_A is f_A/Z_A, it can be shown that equation 3 is equivalent to the integrated form of equation 2 with a constant f_A and f_w and an initial f_F of zero, i.e.:

$$f_F = \{f_w . [D_F/(D_F + D_E + D_R)] + f_A . [D_A/(D_F + D_E + D_R)]\} . \{1 - \exp[-(D_F + D_E + D_R) . t/(V_F . Z_F)]\} \quad (4)$$

The fugacity and the kinetic description of the bioconcentration process are similar in that they are based on the same assumptions, namely that (i) chemical transfer is passive and (ii) the organism is viewed as one, homogeneous compartment. The difference in the two approaches is that in the kinetic approach bioaccumulation is viewed as a balance between the rates of chemical uptake and elimination, whereas in the fugacity approach bioaccumulation is viewed as a process where the chemical is attempting to reach (but not necessarily achieving) a thermodynamic equilibrium. This thermodynamic equilibrium is characterized by equal fugacities of the chemical in the organism, the water and the food consumed by the organism.

The fugacity equations demonstrate that fish-lipid/water partitioning is the driving force of the bioaccumulation process. The strength of the kinetic descriptions is that the rate constants can be measured directly from uptake and depuration experiments. The fugacity-equations, however, distinguish between thermodynamically controlled partitioning phenomena, characterized by the fugacity capacity values (i.e. Z) and pure transport phenomena, described by transport parameters (i.e. D). Fugacity expressions can therefore be useful when investigating the actual mechanism of the bioaccumulation process. The two approaches complement each other, and are best combined. This can be easily achieved by comparing equations 3 and 4, from which it follows that:

$$k_1 = D_F/V_F.Z_W \quad (5)$$

$$k_2 = D_F/V_F.Z_F \quad (6)$$

$$k_A = D_A/V_F.Z_A \quad (7)$$

$$k_E = D_E/V_F.Z_F \quad (8)$$

$$k_R = D_R/V_F.Z_F \quad (9)$$

Equations 3 and 4 show that at infinite exposure time an organism-water bioaccumulation factor, K_B can be defined for an organism simultaneously exposed to contaminated water and food as:

$$K_B = C_F/C_W = (Z_F/Z_W).\{[D_F/(D_F+D_E+D_R)] + [(f_A/f_W).D_A/(D_F+D_E+D_R)]\} \quad (10)$$

or

$$K_B = C_F/C_W = \{k_1/(k_2+k_E+k_R)\} + (C_A/C_W).\{k_A/(k_2+k_E+k_R)\} \quad (11)$$

It also follows from equations 3 and 4 that in food uptake experiments, when organisms are exposed to contaminated food but uncontaminated water, the ratio of organism to food concentrations, i.e., C_F/C_A can be expressed as:

$$C_F/C_A = (Z_F/Z_A).\{D_A/(D_F+D_E+D_R)\} = k_A/(k_2+k_E+k_R) \quad (12)$$

The bioconcentration factor, K_C, which is defined as the ratio of fish and water concentrations at infinite exposure time for organisms exposed to contaminated water only (i.e., $C_A = f_A = 0$), is:

$$K_C = C_F/C_W = k_1 / (k_2 + k_E + k_R) = Z_F/Z_W.\{D_F/(D_F + D_E + D_R)\} \quad (13)$$

Equations 11 to 13 demonstrate that K_B, and K_C are not solely determined by the thermodynamic quantities, i.e., Z_F, Z_W and Z_A, which reflect the affinities of the chemical for the organism, water, and food, but also by the relative rates of chemical uptake from water and food, release to the water, egestion with the faeces, and metabolic transformation. Equation 13 shows that even when no metabolic transformation occurs ($D_R = 0$), the bioconcentration factor only reflects organism-water partitioning when D_E is small compared to D_F.

It thus follows that in order to make reliable predictions about the bioaccumulation potential of hydrophobic chemicals in aquatic organisms and the rate at which bioaccumulation is achieved in organisms, knowledge is required about the processes controlling the exchange of solute between the organism, the water, its food, and faeces.

Deriving Rate Constants from Experimental Data

Equation 3 illustrates that when a contaminated organism is introduced in clean, uncontaminated water (C_w is zero) and consumes uncontaminated food (C_A is zero), it will lose chemicals to the water resulting in a drop of C_F with time. The differential equation describing this process is again equation 1, but with a C_w and C_A of zero, i.e.:

$$dC_F/dt = -(k_2 + k_E + k_R).C_F \qquad (14)$$

which after integration with an initial $C_{F,t=0}$ becomes:

$$C_F = C_{F,t=0}.\{\exp(-(k_2 + k_E + k_R).t)\} \qquad (15)$$

or

$$\ln C_F = \ln C_{F,t=0} - (k_2 + k_E + k_R).t \qquad (16)$$

Equation 16 demonstrates that in a logarithmic plot $\ln C_F$ decreases linearly with time. The slope of this plot is the total depuration rate constant ($k_2 + k_E + k_R$) and has units of reciprocal time.

The rate at which chemicals are being eliminated by organisms can also be expressed by the biological half-time in the organism. This is the time to reach half the initial concentration in the

organism for an organism exposed to uncontaminated water and food. It follows from equation 16 that the biological half-time, $t_{1/2}$ is:

$$t_{1/2} = \ln 2/(k_2 + k_E + k_R) = 0.693/(k_2 + k_E + k_R) \qquad (17)$$

Time to Achieve Steady-State / Equilibrium

The time required to achieve steady state, i.e. a constant ratio between the chemical concentrations in the organism and the water, is of crucial importance in biomonitoring studies since it determines how long a biomonitor should be employed before the concentration in the organism reflects the concentration in the aquatic environment through, for example, the bioaccumulation factor. To determine the time required for a chemical to achieve chemical equilibrium or steady-state, the general model for chemical uptake and depuration in the organism, i.e. equation 1, needs to be revisited. It was demonstrated that this simple model can be used to derive expressions for the chemical concentration in the organism as a function of time. For example, equation 3 and 5 were derived for chemical uptake and depuration. However, it should be realized that these expressions only apply under certain conditions. For example, equation 3 for chemical uptake from water and food is only valid when the organism is exposed to a constant chemical concentration in the water and food. In addition, it applies only when the fish is not significantly growing, lipid levels do not alter, and the rate constants for chemical uptake and depuration do not vary during the exposure period.

Similar to the derivation of expressions for chemical uptake and depuration, it is possible to derive an expression for the time to reach equilibrium or steady-state. Theoretically, chemical

equilibrium or steady-state are achieved only at infinite exposure time. In practice, however, equilibrium or steady-state can be considered to be achieved when the chemical concentration in the organism is 95% of its equilibrium or steady-state value.

The simplest expression is derived for a situation where an organism is exposed to a constant chemical concentration in the water and the food source. If in addition, fish growth, lipid levels and rate constants for chemical uptake and depuration are constant, it can be shown that under those conditions the time to reach 95 % of equilibrium or steady-state, t_{95}, can be expressed as

$$t_{95} = \ln 0.05/(k_2 + k_E + k_B) = 3/(k_2 + k_E + k_B) \qquad (18)$$

Equation 18 illustrates that the time required to reach 95% of equilibrium or steady-state is dependent on the rate constants of chemical depuration. In turn, these rate constants are determined by the properties of the chemical, the organism and the environmental conditions. This will be discussed in more detail in the next section.

Equation 18 can indicate the approximate length of a biomonitoring study, when information regarding the rate constants of chemical uptake and depuration are available. However, its use as a tool to set deployment times and to interpret biomonitoring data in terms of ambient concentrations is limited by the rather stringent conditions of; (1) constant ambient chemical concentrations, (2) no growth, (3) no alterations of lipid levels and (4) constant uptake and depuration rates. When one or more of these conditions do not apply, a different method should be used to

determine the t_{95}. This method requires estimates of the variation of the relevant time-variable-parameters with time. For example, when the biomonitor is a young-of-the-year fish, which is growing, information is needed about the increase of the fish weight with time. And when a mussel is being used to biomonitor ambient chemical concentrations throughout the year, the time variation of the chemical uptake rate constant from the water needs to be known.

The time dependence of relevant parameters affecting the uptake and depuration of contaminants should be incorporated in the calculation procedure to derive t_{95}. Unfortunately, this results in rather complicated expressions. For this reason we are developing a computer-program, ECOTOX. This program determines, to the best of our present understanding of chemical dynamics in aquatic organisms, the relationship between the body burden of the biomonitor and the ambient concentration when environmental conditions and biological properties are changing with time. It also estimates the t_{95} under those conditions. It should be noted, however, that in rapidly changing situations, the t_{95} can loose its significance. In these situations, steady-state and chemical equilibrium can never occur. But more important than t_{95}, is the actual relationship between the body burden and the ambient concentration at any time, which ECOTOX can derive in a user-friendly fashion. However, for ECOTOX to properly relate ambient levels and body burdens reliable information is required about the uptake and depuration rates of chemicals, and how these rates vary with temperature, growth of the organism, altering lipid levels etc. We will now discuss the kinetics of chemical uptake in aquatic organisms and demonstrate the chemical and organism dependence of the uptake and depuration rate constants.

Chemical and Organism Specific Relationships for the Uptake and Depuration of Organic Chemicals in Aquatic Organisms

Lipid-water mass transfer models were derived by Gobas and Mackay (1987) and Mackay and Hughes (1984) to gain further insight into the processes controlling the exchange of chemical between aquatic organisms and water and to develop practical procedures to estimate the bioconcentration kinetics of chemicals in fish. The main feature of this model is that it views the exchange of solute chemical between the water and the organism to take place in a series of aqueous and lipid layers. All transport processes in water phases are therefore grouped together in one overall water phase transport parameter D_w. This overall water phase transport parameter contains all transport parameters $D_{w,i}$ in water phases. The transport parameters $D_{w,i}$ can refer to diffusion, in which case $D_{w,i}$ equals $k.A.Z_w$, where k is the mass transfer coefficient (m/s), A is area of diffusion and Z_w is the chemical's fugacity capacity in the water phase. It can also refer to non-diffusive transport, where the solute is conveyed by a fluid flow G (m_3/s) such that $D_{w,i}$ equals $G.Z_w$. The overall transport parameter D_w can therefore also be expressed as $Q_w.Z_w$, where the transport parameter Q_w (m^3/s) combines all k.A and flow rates G in water phases of the organism.

Similar to D_w all transport processes in lipid phases are combined in an overall lipid phase transport parameter D_L. This lipid phase parameter D_L can be designated $Q_L.Z_L$, where Q_L combines all k.A and flow rates G in lipid phases of the organism and can be considered the "hypothetical lipid flow rate" in the organism. The transport parameters D_w and D_L therefore refer to the net transport of chemical in respectively the water and the lipid phase of the organism.

Assuming that the water and lipid transport processes apply in series it follows that the transport parameter for chemical exchange between the water and the organism D_F can be expressed as:

$$1/D_F = 1/D_W + 1/D_L = 1/Q_W.Z_W + 1/Q_L.Z_L \qquad (19)$$

Since D_F equals $k_1.V_F.Z_W$ or $k_2.V_F.Z_F$ (i.e. equations 5 and 6) and since bioaccumulation is predominantly in the lipids of the organisms. Z_F can be expressed as a fraction L_F of the fugacity capacity in the lipids i.e. as $L_F.Z_L$, where L_F is the lipid fraction of the organism (i.e. V_L/V_F), equation 19 can be rewritten as:

$$1/k_2 = V_L.\{(Z_L/Q_W.Z_W) + 1/Q_L\} \qquad (20)$$

$$1/k_1 = V_L.\{1/Q_W + (Z_W/Q_L.Z_L)\}/L_F \qquad (21)$$

When the lipid-water partition coefficient Z_L/Z_W or K_L is replaced by the 1-octanol-water partition coefficient Z_O/Z_W or K_{OW} (thus assuming Z_L to be equal to Z_O) equations 20 and 21 become:

$$1/k_2 = V_L.\{(K_{OW}/Q_W) + 1/Q_L\} \qquad (22)$$

$$1/k_1 = V_L.\{1/Q_W + (1/Q_L.K_{OW})\}/L_F \qquad (23)$$

This approach may appear to be rather simplistic and the treatment of fish as a series of aqueous and lipid phases seems to ignore the physiology of aquatic organisms. More "sophisticated" models based on gill membrane permeation phenomena or the internal pharmacokinetics can indeed be suggested. However, at this stage, experimental data demonstrating the actual role of membrane permeation and/or the internal pharmacokinetics, on chemical uptake and depuration dynamics do not exist. Since it is questionable to derive models which have a degree of "mechanistic" detail that is not supported by experimental evidence, we prefer the simple model.

The ratios V_L/Q_L and V_L/Q_w can be viewed as the times of chemical transport in V_L m^3 of respectively lipids and water. However, if transport of a given amount of chemical requires a volume V of lipid, it will require a much larger volume i.e. $K_{ow}.V_L$ of water, since the chemical concentration in the water is a factor of K_{ow} lower than in the lipids. The time for the water phase in the organism to transport a certain amount of chemical is therefore K_{ow} times longer than that for the lipid phase. The transport time of the water phase is therefore multiplied with K_{ow} in equation 22 and alternatively the lipid transport time is divided by K_{ow} in equation 23. Since the lipid and water transport processes occur in series these times are additive and the longer time "controls" the bioconcentration kinetics.

The expressions 22 and 23 contain two types of variables namely, (i) biological parameters i.e. V_L, Q_w, Q_L, G_V and L_P, which are specific to a particular organism and its physiological condition and (ii) a chemical parameter K_{ow} expressing the chemical's tendency to partition between lipids and water.

When rate constants or uptake efficiencies have been determined in a particular organism for a series of chemicals, varying in K_{ow}, then equations 22 and 23 can be fitted to the experimental data resulting in values for Q_w and Q_L. The data fitting involves a regression e.g. $1/E_0$ or $1/K_1$ versus $1/K_{ow}$ or $1/k_2$ versus K_{ow}.

Equations 22 and 23 can be successfully fitted to kinetic data in various fish species, resulting in values for Q_w and Q_L. It was found that Q_w increases with increasing body weight of the organism, such that:

$$Q_w = 1.4.M^{0.6} \quad (24)$$

A similar relationship was developed, for Q_L i.e.:

$$Q_L = 0.014.M^{0.6} \quad (25)$$

Theoretical justification for empirical relationships between Q_w and M can be obtained from studies on the influence of oxygen concentration on gill ventilation rates. For example, Norstrom et al. (1976) showed that the gill ventilation volumetric rate G_v in fish is a function of the oxygen concentration in the water C_{ox} (ml of O_2/ml of water), the oxygen transfer efficiency across the gills E_{ox}, the metabolic rate coefficient (ml of $O_2.h^{-1}.g^{-0.8}$) and the body weight of the organism to the power 0.8, i.e.:

$$G_v = A.M^{0.8}/(E_{ox}.C_{ox}) \quad (26)$$

The similarity in body weight dependence of the gill ventilation volumetric rate and Q_w suggests a similarity in these two quantities. More importantly, the similarity in the actual values of Q_w and directly measured or estimated (i.e. equation 26) gill ventilation rates indicates that Q_w is primarily dominated by the gill ventilation volumetric rate. A similar lipid-water mass transfer model can also be developed for chemical uptake from consumed food and elimination to faecal matter in the GI-tract. The derivation of this model can be found in Gobas et al. 1988. It was shown that similar to equations 22 and 23 chemical-specific relationships can be derived for the rate constants k_1 and k_2 and the chemical uptake efficiency from food (E_D) i.e.:

$$1/k_1 = (V_F/G_T).\{(G_D.L_G/Q_{WF}).K_{ow} + (G_D.L_G/Q_{LF}) + 1\} \quad (27)$$

$$1/k_E = (V_F.L_F/G_O.L_G).\{(G_O.L_G/Q_{WF}).K_{OW}+(G_O.L_G/Q_{LF})+1\} \quad (28)$$

$$1/E_O = (G_O.L_G/Q_{WF}).K_{OW} + G_O.L_G/Q_{LF} + 1 \quad (29)$$

where G_T is the volumetric feeding rate (in m^3 food per hour), G_O is the volumetric egestion rate (in m^3 faeces per hour), Q_{WF} is the water phase transport parameter for chemical exchange between the GI-tract and the final storage site in the organism (in m^3 per hour), Q_{LF} is the lipid phase transport parameter for chemical exchange between the GI-tract and the final storage site in the organism (in m^3 per hour) and L_G is the organic or "lipid" fraction of the gastro-intestinal contents (in grams of organic matter per gram of GI contents).

Equation 29 demonstrates that by experimental measurement of E_O for a series of chemicals with varying K_{OW} under controlled conditions, i.e., a constant feeding rate and no uptake of chemical from the water, it is possible to determine the fundamental kinetic parameters Q_{WF} and Q_{LF}. Knowledge of these parameters is invaluable for a reliable estimation of organic chemical bioaccumulation from contaminated food.

Gobas et al. (1988) showed that experimental data for dietary uptake of chemicals in fish fit this simple relationship with values for $(G_O.L_G/Q_W).K_{OW}$ of 5.3 (+/- 1.5).10^{-8} and for $(G_O.L_G/Q_L + 1)$ of 2.3 (+/- 0.3), thus resulting in the following relationship for E_O, k_A and k_E

$$1/E_O = 5.3.10^{-8}K_{OW} + 2.3 \quad (30)$$

$$1/k_A = (V_F/G_T).(5.3.10^{-8}K_{OW} + 2.3) \quad (31)$$

$$1/k_e = (V_F.L_F/G_o.L_G).(5.3.10^{-8}K_{ow} + 2.3) \quad (32)$$

The relationships that have been presented above for the chemical uptake and elimination kinetics have been experimentally validated in several species of fish (Gobas and Mackay 1987, Gobas et al. 1988 & 1989). However, for other aquatic biomonitors such as mussels, mayfly and aquatic plants the above relationships for the uptake and elimination rate constant have yet not been established.

The following chapters report experiments which were designed to determine the rate constants for chemical uptake and elimination in mussels, mayflies and aquatic plants.

The Use of Kinetic Rate Constants in Biomonitoring

We will now demonstrate with an example how kinetic rate constants can be used to set deployment schemes for aquatic biomonitors and to translate chemical concentrations in the biomonitor to concentrations in the water. Before a biomonitor can be used as a tool to measure chemical concentrations in the water the chemical uptake and elimination kinetics should be established. Preferably, this is achieved by performing uptake and elimination experiments in the field, which is often very difficult, or in the laboratory. Preferably, these experiments should involve the chemicals that are going to be the subject of the monitoring program. This procedure, which can be viewed as the "calibration" of the biomonitor, will establish the rate constants for uptake and elimination of each chemical of interest. Fish are among the species, for which most calibration data are available. We will therefore illustrate the case of the calibration data for

biomonitoring with fish. For this purpose we will use a 5 gram fathead minnow (V_F is 0.005 L)with a lipid content of 6 %, as a biomonitor. The biomonitor will be deployed in a cage to monitor chemical exposure from the water. We will assume that the fish is contaminant-free at the time of deployment. To derive the rate constants for chemical uptake from and elimination to the water i.e. k_1 and k_2 equations 22 to 25 can be used. Equations 24 and 25 show that the Q_W and Q_L for the fathead minnow are respectively $1.4*5^{0.6}$ i.e. 3.7 L/d and $0.014*5^{0.6}$ i.e. 0.037 L/d. Substitution of Q_W and Q_L in equations 22 and 23 then results in:

$$1/k_1 = 0.00136 + 0.136/K_{OW} \quad (33)$$

$$1/k_2 = 8.1.10^{-5}.K_{OW} + 0.0081 \quad (34)$$

Equations 33 and 34 demonstrate that for trichlorobenzene (TCB) with a log K_{OW} of 4.0, k_1 is 728 d^{-1} and k_2 is 1.2 d^{-1}. For mirex with a log K_{OW} of 7.5 these rate constants are respectively 735 d^{-1} and 0.0004 d^{-1}.

The rate constants for chemical uptake from food and elimination to the faeces i.e. k_A and k_E can be derived from equations 31 and 32. If the caged fathead minnow feeds at a rate of 1 % of its own body weight per day, k_A for TCB is 0.0043 d^{-1} and for mirex is 0.0025 d^{-1}. If the faecal egestion rate G_O is one-third of the feeding G_I and that L_F and L_G are approximately equal equation 32 shows that k_E can be calculated to give values of 0.0014 d^{-1} for TCB and 0.00083 d^{-1} for mirex.

When we assume no metabolic transformation of TCB and mirex in the fish the total depuration rate constant k_T (i.e. the sum of k_2, k_E and k_G) can be determined as (1.2+0.0014) i.e. 1.2014 d^{-1} for TCB and (0.0004 + 0.00083) i.e. 0.00123 for mirex. These calculations

demonstrate that for TCB, chemical elimination is predominantly to the water and that chemical elimination to the faeces is unimportant. For mirex the situation is quite different with 2/3 of its body burden being eliminated in faecal matter and only 1/3 to the water.

Comparison of the uptake rate constants k_1 and k_A shows that for TCB, chemical uptake from the food will become a significant route of exposure when the chemical concentration in the food C_A is approximately 150,000 times higher than that in the water. In that case the chemical uptake rate from the water $k_1.C_w$ would equal the uptake rate from the food $k_A.C_A$, i.e. water and food are equally important exposure routes. For a chemical with a K_{ow} of only 10,000 it is extremely unlikely to have concentrations in food sources that exceed the chemical concentration in the water by 150,000. A reasonable estimate of the chemical concentration in the food can be derived by assuming a chemical equilibrium of the chemical in the food with that in the water i.e. C_A equals $0.05.K_{ow}.C_w$ (Mackay 1982). For TCB, this implies a C_A which is only 500 times larger than C_w. It can thus be concluded that for TCB, gill ventilation of water is the main exposure route. The body burden of the biomonitor thus reflects the concentration in the water. The relationship between the concentration in the organism and the water can now be established by substituting the calculated (or measured) values for k_1, k_2, k_E, k_A and k_R in equation 11 i.e.:

$$K_B = C_F/C_w = \{728/(1.2+0.0014+0)\} + \{0.05.10^4.(0.0043/(1.2+0.0014+0))\} = 607 \qquad (35)$$

It thus follows from equation 35 that C_w equals the measured concentration in the biomonitor divided by 607 i.e. $C_F/607$. However, it should be noted that this procedure is only valid when

the organism and water are at steady-state at the time of sampling. In practice, this steady state will be practically reached after $3.0/(k_2 + k_E + k_R)$ i.e. 2.5 days assuming a chemical concentration which does not vary in time. The fish should thus be employed for at least 3 days before the simple correlation of $C_F/607$ can be used to derive the chemical concentration in the water.

For mirex the situation is again quite different. With values for k_1 and k_A of respectively 735 d^{-1} and 0.0025 d^{-1} it follows that the chemical concentration in the food of the organism has to be approximately 300,000 higher than the concentration in the water before food and water are equally important exposure routes for the fish. However, for a chemical with a K_{ow} of 32,000,000, simple partitioning of the chemical in the food source of the organism may cause such a large difference between chemical concentrations in the water and food. Assuming equilibrium partitioning of mirex between the food and the water results in an estimate of C_A, which is $0.05.K_{ow}$ i.e. 1,600,000 fold higher than C_w. In that case 84% of the total chemical exposure is chemical uptake from food and only 16% of the body burden of the organism is chemical taken up from the water. It thus follows that chemical uptake from food is the main exposure route for mirex in the fish.

The body burden of mirex in the fish therefore most closely reflects the concentration in the food source, not the water. If the fish is used to monitor the chemical concentration in the water or the food, it is necessary to know either the chemical concentration in the water or in the food, or the relationship between the chemical concentrations in the food and water. Since the main purpose of the biomonitor is to "measure" the chemical concentration in environmental compartments such as the water and food, this seems to contradict the purpose of the biomonitor.

However, the body burden C_F for mirex is the function of two parameters i.e. C_A and C_W. Different combinations of the parameters C_W and C_A can therefore result in the same C_F. In order to translate body burdens into concentrations in the water it is necessary to know C_A or to make an estimate about the value for C_A. As discussed earlier, $0.05.K_{OW}.C_W$ is often a reasonable estimate for C_A. It then follows that K_B can be calculated as:

$$K_B = C_F/C_W = \{735/(0.0004 + 0.00083 + 0)\} + \{0.05.10^{7.5}.(0.0025/(0.0004 + 0.00083 + 0))\} = 9.2.10^5 \quad (36)$$

Equation 36 thus shows that C_W can be calculated as $C_F/9.2.10^5$. The time required to reach this steady state is condition is approximately $3.0/(k_2 + k_E + k_B)$ i.e. 2440 days. This shows that for mirex the biomonitor has to be deployed for a much longer time than when TCB is being monitored. It may even be possible that the biomonitor will never reach this steady state condition within its life time. The body burden for mirex in the biomonitor is thus a function of the deployment time t. This time function is given by equation 3, which after substitution of the values of the rate constants is:

$$C_F = \{735.C_W/(0.0004 + 0.00083 + 0)\} + \{0.05.10^{7.5}.C_W.(0.0025/(0.0004 + 0.00083 + 0))\}./1\text{-}\exp(-[0.0004 + 0.00083 + 0].t) \quad (37)$$

The example discussed above shows the necessity to interpret biomonitoring data on a chemical-specific basis. It can thus be concluded that when biomonitors are to be used as a tool to measure chemical concentrations in the water and chemical exposure, it is important to be know how uptake and depuration kinetics are dependent on chemical properties of the chemicals of interest.

This example illustrates the role of rate constants of chemical uptake and depuration in determining the relationship between the chemical body burden in the biomonitor and the ambient concentration. It thus transpires that in order to predict chemical body burdens in aquatic organisms resulting from chemical exposure in the environment, or to determine ambient levels from body burdens in biomonitor, it is crucial to have information regarding the (1) the rate constants of chemical uptake and depuration in various organisms/biomonitors (2) how the rate constants vary with chemical properties, biological characteristics and environmental conditions, (3) the mechanism underlying chemical uptake and depuration for (predictive) modelling. It is one of the objectives of this research program to provide information on these issues.

CHAPTER 4

CALIBRATION OF THE FRESH WATER MUSSEL *ELLIPTIO COMPLANATA* FOR QUANTITATIVE BIOMONITORING OF HEXACHLOROBENZENE AND OCTACHLOROSTYRENE IN AQUATIC SYSTEMS

Introduction

The fresh water mussel *Elliptio complanata* has been frequently used to monitor organic contaminant exposure in the Great Lakes (Curry 1977/78; Kauss and Hamdy 1985; Pugsley et al. 1985; Muncaster 1987). However, in order to translate observed body burdens in the mussel to chemical concentrations in the water, the relationship between chemical concentrations in the water and the mussel should be established. This relationship is controlled by the uptake and elimination kinetics of chemicals in the mussel. This study reports a bioconcentration experiment in *Elliptio complanata* for hexachlorobenzene and octachlorostyrene. It demonstrates: (i) the derivation of uptake and elimination rate constants from the experimental data; (ii) the use of kinetic rate constants in establishing chemical-specific relationships for chemical concentrations in the mussel and the water; and (iii) the role of these relationships in the planning and interpretation of biomonitoring studies.

Materials and Methods

Hexachlorobenzene (HCB) was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin and octachlorostyrene (OCS) was obtained from the Foxboro Co., South Norwalk, Connecticut.

Glass distilled acetonitrile and petroleum ether were from BDH Inc., Toronto, Ontario. Pesticide grade n-hexane was from Caledon Laboratories Ltd., Georgetown, Ontario. Florisil (60-100 mesh) was from Supelco Canada Ltd., Oakville, Ontario. Anhydrous sodium sulphate from J.T. Baker Chemical Co., Phillipsburg, New Jersey was heated to 650°C overnight and stored at 130°C before use.

Mussels (*Elliptio complanata*) were from Balsam Lake, Ontario (44°23'N, 78°50'W), and held in 700 litre tanks until used experimentally. Windsor tap water (pH=7.4, hardness=104 ug/L, dissolved oxygen=12 ug/L) was continuously filtered, aerated, and held at 10°C. The 69 mussels used in the experiment averaged 6.02 (± 0.30) grams in weight and 67.4 (± 1.0) cm in length. Analysis of collected mussels before the start of the experiment showed no detectable levels of HCB and OCS.

The uptake phase of the experiment was performed in a continuous flow apparatus similar to that used by Bruggeman et al. (1984), Opperhuizen et al. (1985) and Gobas et al. (1989). It consisted of a 100 litre glass tank filled with dechlorinated Windsor tap water at 20°C. To contaminate the water, an Asti teflon pump (Cole-Parmer Instrument Co., Chicago, Illinois) circulated water through a generator column and a glass fish tank at 60 litres per hour. All fittings and tubing were of teflon and glass. The generator column was prepared by dissolving 150 mg of HCB and OCS in hexane. The hexane was evaporated from the solution onto glass wool, resulting in "coating" the glass wool with HCB and OCS. The contaminated glass wool was placed at the bottom of a one litre column and overlain with hexane rinsed glass wool. The water was equilibrated for 3 weeks before mussels were added. Three mussels and two water samples were taken daily for 11 days. All mussels were shucked and weighed when sampled, then frozen

immediately.

The elimination phase was performed in a 50 litre glass tank. The water was filtered continuously by an Eheim activated carbon power filter at a rate of 270 litres per hour. Mussels were sampled in triplicate for 11 days in the same manner as previously described.

Individual mussels were homogenized for approximately 1 minute in a mixture of 120 ml of acetonitrile and 40 ml distilled, deionized water using a Brinkman Polytron (Sybron Canada Ltd., Rexdale, Ontario). The mixture was filtered by suction through a Whatman no. 1 glass filter and the liquid phase collected for liquid-liquid extraction in petroleum ether. The filtered aqueous phase was extracted three times in a total of 300 ml of petroleum ether. The organic phase was separated and passed through a column containing 30 grams sodium sulphate (for drying), then concentrated to a five ml volume in a Kuderna-Danish (K-D) evaporator. This extract was passed through a 20 g florisil column and eluted with 200 ml petroleum ether to remove lipids. This extract was concentrated and analyzed by gas chromatography.

Water samples of 500 ml were collected daily during the uptake phase and were extracted three times in a total of 300 ml petroleum ether. The extract was dried by passing it through a sodium sulphate column, then concentrated on a K-D evaporator and analyzed by gas chromatography.

Gas chromatographic analysis was performed on a Hewlett-Packard 5790A (Hewlett-Packard Canada Ltd., Mississauga, Ontario) equipped with a DB-5 capillary column (J&W Scientific, Folsom CA), a ^{63}Ni electron capture detector, a Hewlett-Packard auto-injector

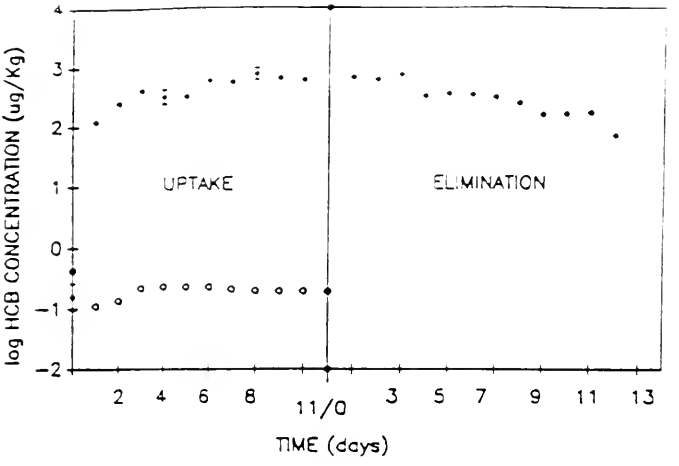
and a Hewlett-Packard 3390A integrator. Injector temperature was 250°C, detector temperature was 300°C, and column temperature was programmed from 50 to 250°C. Carrier gas was ultra high purity helium at 1.5 ml/min. Make-up gas was 5% methane-95% argon at 60 ml/min, and the injection mode was splitless. Injection volume was 1 uL.

Average lipid content of the mussels was determined by homogenizing 10 clams in a blender in a solution of 60% chloroform and 40% methanol. The mixture was filtered by suction through a Whatman no. 1 glass filter and the solvent was collected for evaporation after which the lipids were determined by weight.

During the progress of this research, the Great Lakes Institute participated in quality assurance programs with both the Canada Wildlife Service and the International Joint Commission. At all times the laboratory was found to provide accurate, precise results. Unfortunately it was not possible to develop a laboratory exchange program with the Ministry of the Environment, Rexdale Laboratory. As both laboratories have, however, participated in the International Joint Commission quality assurance check, it is probable results are compatible between the two laboratories.

Results and Discussion

Figure 4.1 illustrates the observed concentrations of HCB and OCS in the water and in the mussel as a function of the exposure time. It shows that the initial concentrations of HCB (0.43 ug/L) and OCS (0.16 ug/L) in the water are below the reported aqueous solubilities of the two chemicals, i.e. 4.69 ug/L for HCB (Miller et al. 1985) and 2.5 ug/L (Bjerk et al. 1980) for OCS. This suggests that HCB and OCS were truly dissolved. After the mussels were added, the HCB and OCS concentrations in the water dropped



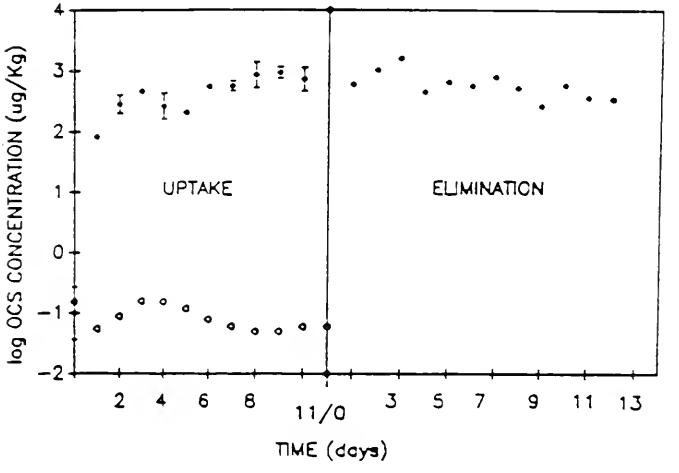


FIGURE 4.1

Logarithms of the concentration of HCBs and OCS in the mussel during the uptake and depuration period.

rapidly due to the high initial uptake rates in the mussels. HCB and OCS concentrations in the water restored after 3 to 4 days to approximately half the initial water concentration and remained approximately constant throughout the remainder of the uptake period. At the end of the exposure period a steady-state appears to have been reached for HCB but not for OCS. During the depuration phase, when the mussels were exposed to uncontaminated water, concentrations of HCB and OCS in the mussels declined logarithmically over time. This suggests that the kinetics of these chemicals can be well described with a simple mussel-water two compartment model with first order rate constants using the following differential equation:

$$dC_M/dt = k_1.C_w - k_2.C_M \quad (1)$$

where C_M is the chemical concentration in the mussel, C_w is the concentration in the water, t is time and k_1 and k_2 are the rate constants for chemical uptake from the water and elimination to the water. If the chemical concentration in the water is constant, equation 1 can be integrated to give:

$$C_M = C_w.(k_1/k_2).(1-\exp(-k_2.t)) \quad (2)$$

The elimination rate constant k_2 was thus determined from the slope of the log C_M versus time plot as $2.303.d(\log C_M)/dt$. This resulted in values of 0.178 (± 0.036) d^{-1} for HCB and 0.070 (± 0.045) d^{-1} for OCS, where the values within brackets are 95% confidence intervals. This corresponds to half-life times of respectively 3.9 and 9.9 days and demonstrates that chemicals with higher K_{ow} (i.e. log K_{ow} of HCB and OCS are respectively 5.45 and 6.29) have higher half-life times in the mussel. This corresponds to observations by Pruel et al. (1986) in the blue mussel *Mytilus*

edulis and by Gobas et al. (1989) in fish. It thus follows that to reach a steady-state for OCS, mussels should be deployed for a longer period of time than for HCB.

Since during the uptake phase the HCB and OCS concentrations in the water were not constant and the uptake phase was too short for OCS to reach a steady-state, k_1 and BCF can not be simply deduced from the experimental data using equation 2 or calculated by available computer programs for data fitting such as BIOFAC (Blau and Algin 1978). To derive the correct values for k_1 and BCF under these conditions, Gobas et al. (1989) suggested performing a numerical integration procedure. This procedure involves the calculation of increments in mussel concentrations dC_M over time intervals dt from equation 1, i.e.:

$$dC_M = (k_1.C_w - k_2.C_M).dt \qquad (3)$$

where dt was chosen to be 0.04 d, k_2 was 0.178 d^{-1} for HCB and 0.07 d^{-1} for OCS and C_M is the sum of all previous dC_M, i.e., dC_M. The chemical concentration in the water C_w at every exposure time t, i.e., dt, was estimated by fitting the observed water concentrations to a series of linear functions, which each connect the observed water concentration data at two consecutive exposure times. The uptake rate constant k_1 was then selected to produce the best agreement between calculated and observed concentrations in the mussel. The best fit of the observed data was the one with the k_1 value, for which the sum of the squared differences between calculated and observed concentrations in the mussel was the smallest. The uptake rate constants were determined to be 650 d^{-1} for HCB and 1010 d^{-1} for OCS.

The bioconcentration factors (BCFs) of HCB and OCS can now be

calculated as k_1/k_2 and are respectively 3,650 and 14,500. The k_1 and BCFs calculated following this procedure are not affected by the duration of the uptake period or by variations in the concentration in the water. These BCFs can also be expressed on a lipid weight basis, as the ratio of the chemical concentrations in the lipid tissue of the mussel and the water, i.e., K_L, by dividing the BCFs by the lipid fraction of the mussels, i.e., 0.0084. This results in log K_L values of 5.64 for HCB and 6.24 for OCS, which correspond excellently with the reported log K_{ow} values of 5.45 for HCB and 6.29 for OCS. This demonstrates that the 1-octanol-water partition coefficient is an excellent predictor of the bioconcentration factor of these chemicals in *Elliptio complanata*.

Studies by Roesijadi et al. (1978a and 1978b) and by Pruel et al. (1986) in *Mytilus edulis* suggest that water is the predominant source for PAH and PCB bioaccumulation and that uptake of chemical from particulate matter is a relatively insignificant route of exposure. This is further supported by field studies, in which *Elliptio complanata* was deployed at particulate rich and poor sites (Muncaster 1987). If the water is indeed the major source for uptake and bioaccumulation of HCB and OCS, *Elliptio complanata* can be used to monitor the concentrations of these chemicals in the water. Measured steady-state concentration in the deployed mussel can simply be related to the chemical concentrations in the water through the BCF or K_{ow}, i.e., C_w equals $C_M/(L_M.K_L)$ or $C_M/(L_M.K_{ow})$.

The length of time, for which the mussels should be deployed to reach 95% of steady-state, is $-\ln 0.05/k_2$ or $3.0/k_2$. For HCB this means that the mussels should be deployed for 17 days and for OCS 43 days. Deployment times of 21 days (Kauss and Hamdy 1985) and 40 days (Pugsley et al. 1985, Muncaster 1987) may therefore be sufficient to reach equilibrium while monitoring of HCB, but too short for OCS.

It can be concluded that when organisms are to be used as a tool to measure chemical concentrations in water, they have to be "calibrated". This can be achieved by determining the uptake and elimination kinetics of the chemicals of interest in a laboratory experiment, but preferably in the field. As demonstrated for HCB and OCS in the mussel, this results in practical relationships between chemical concentrations in the organism and the water and an appropriate duration of deployment.

PATTERNS OF ORGANIC CONTAMINANT ACCUMULATION BY FRESHWATER MUSSELS IN THE ST. CLAIR RIVER

Introduction

Because of their sedentary nature, bivalve molluscs are particularly useful in detecting spatial variation in contaminant levels (Broman and Ganning 1986, Foster and Bates 1978, Greig and Sennefelder 1985). More recent studies have involved the deployment of contaminant-free bivalves for a fixed period of time in the habitats under study (Kauss et al. 1981, Kauss and Hamdy 1985). From the studies it was evident that different compounds require different exposure times for body burdens to reach chemical equilibrium. As noted in the previous section it is possible to calibrate mussels to predict the time required to attain chemical equilibrium with their environment.

The study presented in this section examines three aspects of organic contaminant uptake by *Elliptio complanata* (Lightfoot), at four sites along the lower St. Clair River in 1986 and 1987. In addition to measuring the length of exposure required to approach equilibrium body burdens, the extent of temporal heterogeneity, and the impact of position in the water column on contaminant accumulation were investigated. The following study addresses these questions with specific reference to three compounds with known point sources in the Huron-Erie corridor: pentachlorobenzene (QCB), hexachlorobenzene (HCB), and octachlorostyrene (OCS) (Oliver and Pugsley 1986; Kauss and Hardy, 1985). The octanol-water partition coefficients (K_{ow}) of these compounds range over one order of magnitude with log K_{ow} values of 6.3, 5.5 and 4.9 for OCS, HCB, and QCB respectively (Oliver 1987).

Methods

Mussel Collection and Deployment

Elliptio complanata, ranging in size from 6.5 to 7.2 cm, were collected from Balsam Lake near Lindsay, Ontario and held in 1000 litre tanks at 10° C until their deployment. Pentachlorobenzene and OCS levels were below detection limits in mussels from Balsam Lake, while trace amount of HCB (approx. 0.1 ng/g) were detected in two of six samples.

Specimens were placed in wire-mesh cages (Kauss and Hamdy 1985), for 20, 40 and 86 day intervals 1986 at three sites along the St. Clair River (Walpole Island, Chenal Ecarte, and Wallaceburg; see Figure 4.2.

To assess the influence of vertical position in the water column on contaminant exposure, mussels were suspended near the surface as well as on the sediment. An additional cage was placed approximately one metre above the sediment-water interface at the Walpole and Chenal Ecarte sites. Deployment began on July 11, 1986, with a 28 day lag at the Wallaceburg site resulting from vandalism. The sediment cage at Chenal Ecarte was disturbed, resulting in no data for the first four exposure periods.

In 1987, mussels were deployed for 21, 42, 63, and 84 days at the Walpole and Chenal Ecarte sites and among aquatic vegetation adjacent to the Chematogan Channel ("Marsh", Figure 4.2). All cages were placed approximately one metre below the water surface. In order to examine the extent of seasonal variation in contaminant accumulation, initial deployment on June 8, 1987, was followed by a second cycle of experiments with similar exposure periods

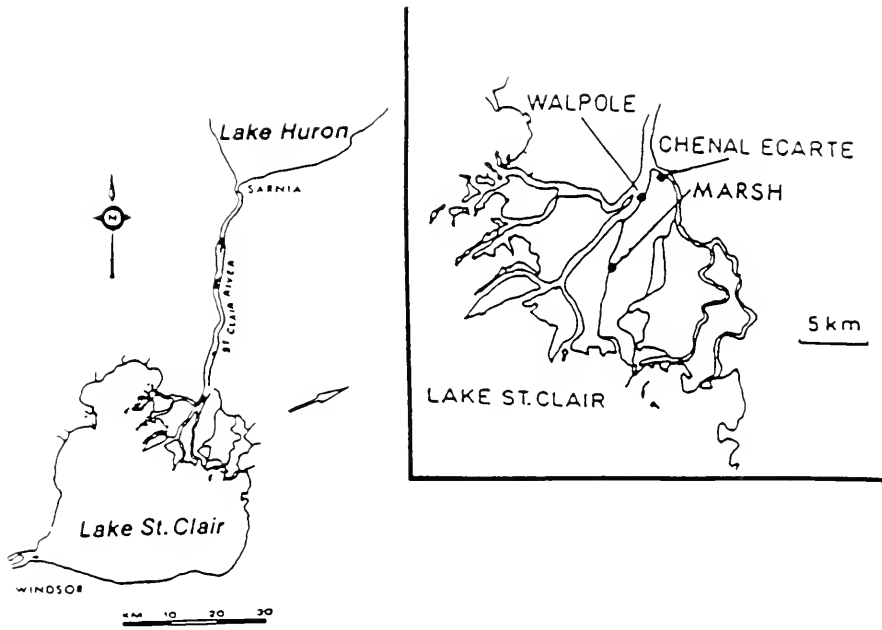


Figure 4.2

Location of the four sampling sites in the St. Clair delta.

beginning on August 31, 1987. A 21 day delay in the experiment occurred at the Marsh site, also a result of vandalism. In addition to these long term studies, three mussels were exposed at each site for twenty-four consecutive one week periods to gain insight into the temporal heterogeneity encountered in natural ecosystems.

A minimum of three specimens for a given exposure period from each site were analyzed to assess individual variability of uptake. As laboratory time permitted, more than three mussels were analyzed for selected exposures in 1986. The exact number of replicates for each treatment are given in the applicable tables. After exposure, specimens were immediately shucked, allowing excess fluid to drain, and wrapped in hexane rinsed aluminum foil. Samples were frozen at -20° C until analyzed.

Analytical Techniques for Organic Contaminants

The extraction technique was similar to that reported by Muncaster et al. (1988). The limits of quantification for QCB, HCB and OCS were 0.2 ng/g. Recovery values for mussel tissues ranged from 87 - 94 % for the study compounds. No corrections were made for recovery efficiencies. All concentrations are expressed as ng/g wet weight. Body burdens can be converted to a dry weight basis by multiplying them by 9.09. Lipid content (expressed as a percent of dry weight) of fifteen *E. complanata* specimens ranged from 1.8 to 3.4% (mean = 2.6%, S.E. = 0.5%). Possible spatial and seasonal variation in lipid content might occur among individuals (Boon et al. 1985), and should be considered when comparing seasonal differences in contaminant uptake. Factorial ANOVAs with replication were used to analyze the differences in body burdens among cage positions, consecutive one week exposures, exposure

periods, and sites. The confidence intervals represent ± one standard error.

Results

Differences in Body Burden Among Depths

There were no significant differences ($p < 0.05$) in the body burdens of QCB, HCB, or OCS in mussels placed in cages near the surface, one metre from the sediment, and on the sediment at the Walpole and Chenal Ecarte sites in 1986 with the exception of QCB at Chenal Ecarte (see Figure 4.3 and Figure 4.4). Similarly at Wallaceburg, no significant differences were detected in body burdens for any of the three contaminants between mussels suspended near the surface and those deployed on the sediment (see Figure 4.5)

Contaminant Accumulation at Single Sites

Chemical uptake was confirmed to be chemical specific and discussed in Chapter 3. Octachlorostyrene displayed a relatively linear increase in concentration with time at the four sites (Figures 4.3, 4.4, 4.5, 4.6), to maximum mean levels of 19.6 and 6.2 ng/g for 1986 and 1987 respectively. Levels of HCB also increased with exposure time, however, body burdens appeared to reach steady state in 1986 at maximum levels of 4 ng/g after 21 days. HCB concentrations in 1987 were approximately half those in 1986. Levels of QCB were below that of HCB or OCS with a mean concentration of 0.4 ng/g in 1986 and 1987 (Figures 4.3, 4.4, 4.5.

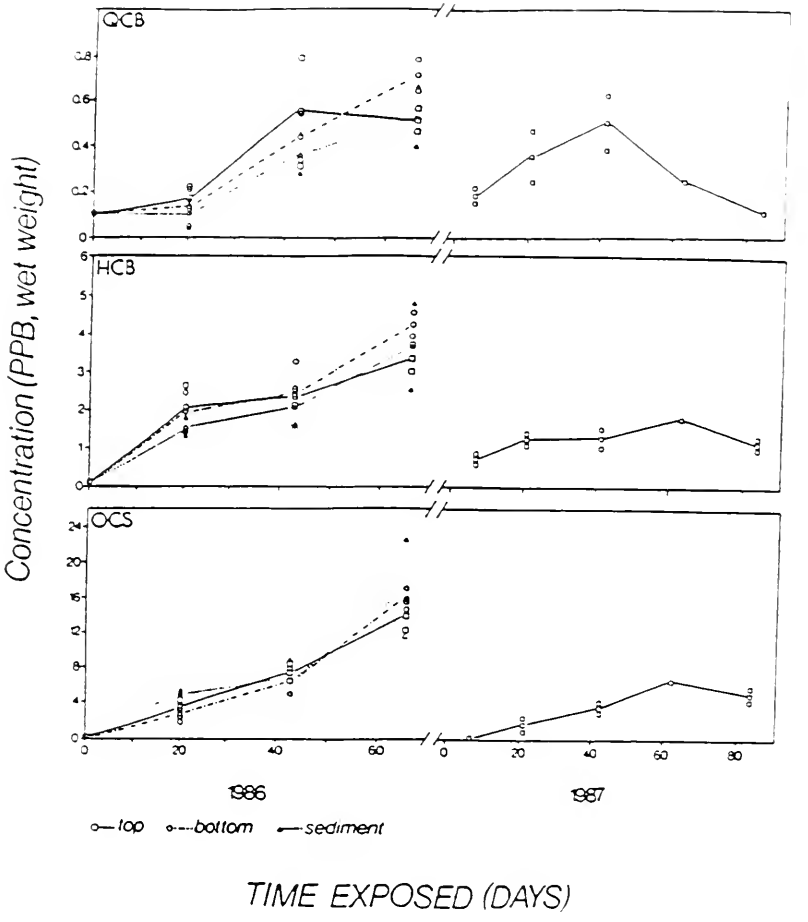


Figure 4.3

Mean accumulation of QCB, HCB, and OCS in *Elliptio complanata* exposed at the Walpole site in 1986 (July 11 to September 13) and 1987. Outer symbols represent ± 1 standard error.

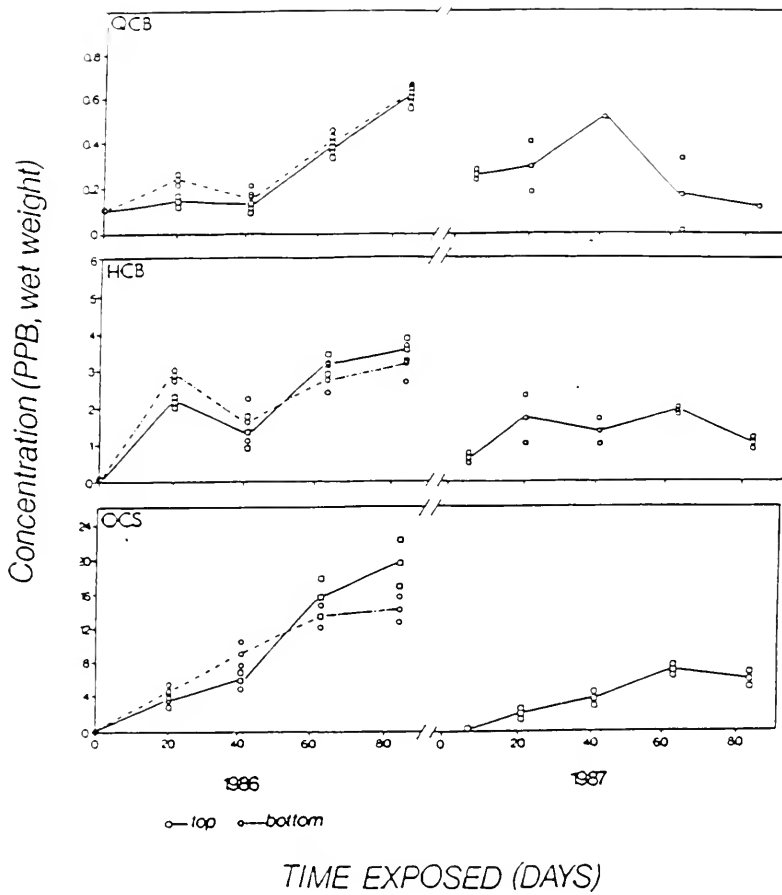


Figure 4.4

Mean accumulation of QCB, HCB, and OCS in *Elliptio complanata* exposed at the Chenal Ecarte site in 1986 and 1987. Outer symbols represent ± 1 standard error.

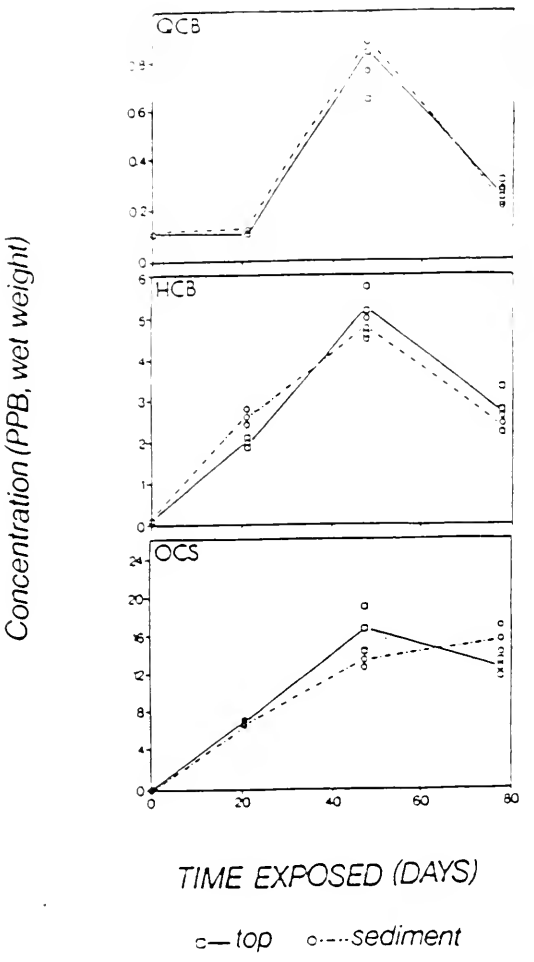


Figure 4.5

Mean accumulation of QCB, HCB, and OCS in *Elliptio complanata* exposed at the Wallaceburg site in 1986. Outer symbols represent ± 1 standard error.

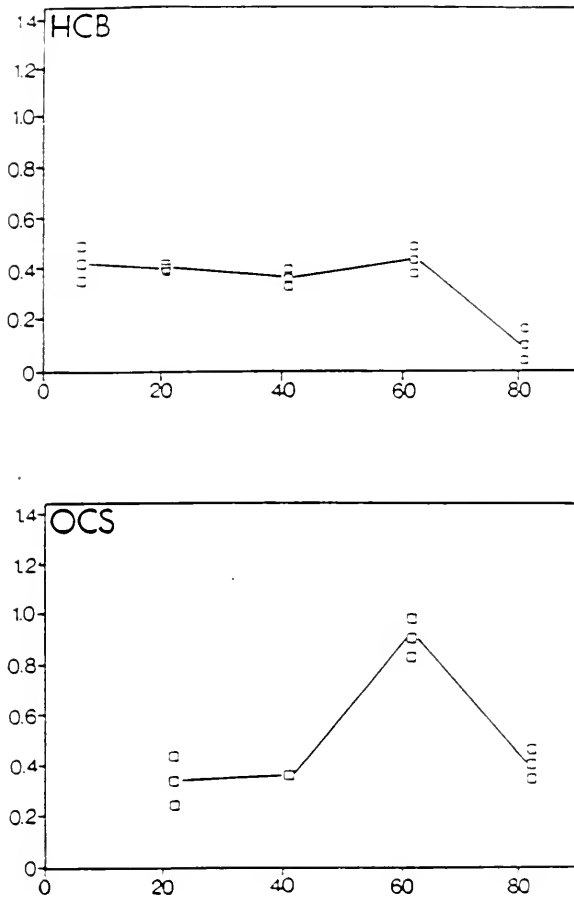


Figure 4.6

Mean accumulation of HCB, and OCS in *Elliptio complanata* deployed at the Marsh site in 1987. Outer symbols represent ± 1 standard error.

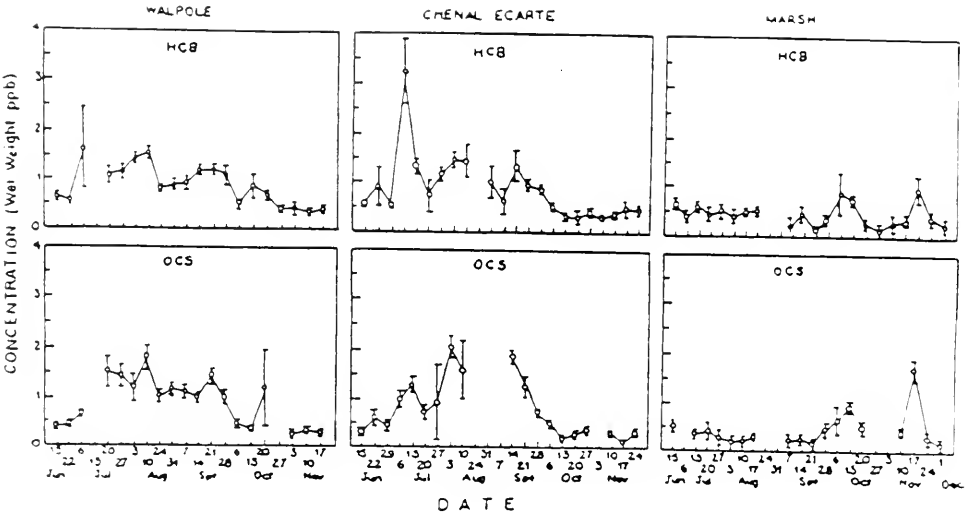


Figure 4.7

Mean body burdens (± 1 S.E.) of HCB and OCS in *Elliptio complanata* mussels deployed for twenty four consecutive one week periods in 1987 at the Walpole, Chenal Ecarte and Marsh sites.

Variation in Contaminant Accumulation Among Sites

No significant differences were observed in the levels of QCB or OCS (pooled over depth) in mussels at the Walpole and Chenal Ecarte sites for seven exposure periods in 1986. Concentrations of HCB were, however, significantly greater at Chenal Ecarte than at Walpole in 1986 although similar levels were observed at the two sites in 1987. Direct comparisons among the three sampling sites could not be made due to staggered starting dates. Contaminant accumulations at Wallaceburg were, however, similar to those at Walpole and Chenal Ecarte (Figures 4.3, 4.4, 4.5). For example, mean concentrations of HCB and OCS (pooled over depths) for 43 - 48 day exposures in August, 1986 were 3.9 and 14.6 ng/g for Walpole and Chenal Ecarte combined versus 4.9 and 15.2 ng/g for Wallaceburg. Contaminant levels at Wallaceburg did not increase between the last two samplings in 1986. Mussels deployed at the Marsh site in 1987 accumulated lower levels of HCB and OCS (0.4 and 0.9 ng/g respectively) than those at Walpole and Chenal Ecarte (Figure 4.6). No detectable levels of QCB were observed at the Marsh site.

Seasonal Variation in Contaminant Accumulation

The mussel deployment and recovery schedules permitted a comparison of contaminant accumulation between exposures of fixed length but at different periods of the summer and fall. In 1986, the pooled (all depths combined) mean body burdens for all three contaminants differed significantly among the starting dates for twenty and 41 day exposure periods at both the Walpole and Chenal Ecarte sites (Table 4.1). For all compounds measured at Walpole and Chenal Ecarte in 1986, the greatest accumulation of contaminants occurred in the latter part of the year. Although HCB

Table 4.1

Seasonal variation in accumulation of contaminants at Walpole and Chenal Ecarte sites in 1986. Mean body burdens (± 1 S.E.) represent individuals pooled from all three cage positions. Tests are for significant differences among starting dates.

				Concentration (ng/g, Wet Weight)		
Days of Exposure	Starting Date	Site	N	QCB	HCB	OCS
20	Jul 11	Walpole	6	0.15 (0.03)	1.99 (0.34)	3.05(0.55)
		C. Ecarte	6	0.18 (0.03)	2.48 (0.17)	4.16 (0.47)
20	Jul 31	Walpole	3	0.09 (0.03)	1.51 (0.23)	5.02 (0.33)
		C. Ecarte	3	0.23 (0.10)	1.83 (0.19)	4.60 (0.83)
21	Sep 12	Walpole	5	0.55 (0.06)	3.10 (0.29)	7.16 (1.60)
		C. Ecarte	9	0.56 (0.11)	3.48 (0.36)	8.30 (1.53)
Significance		Walpole		***	*	*
		C. Ecarte		*	*	*
41	Jul 11	Walpole	6	0.12 (0.02)	1.83 (0.30)	6.60 (1.20)
		C. Ecarte	6	0.15 (0.03)	1.49 (0.33)	7.50 (1.04)
43	Jul 31	Walpole	9	0.45 (0.09)	2.31 (0.29)	6.92 (0.76)
		C. Ecarte	3	0.36 (0.04)	3.00 (0.27)	8.79 (0.49)
43	Aug 21	Walpole	16	0.63 (0.03)	3.75 (0.19)	14.47 (0.67)
		C. Ecarte	19	0.73 (0.07)	4.13 (0.19)	15.22 (0.36)
Significance		Walpole		***	***	***
		C. Ecarte		***	***	***

* $p < 0.05$ *** $p < 0.001$

Table 4.2

Seasonal variation in mean body burdens (1 S.E.) of HCB and OCS at Walpole and Chenal Ecarte sites in 1987. Three mussels were analyzed except where indicated. Tests are for significant differences among starting dates.

		Concentrations (ng/g, Wet Weight)			
Days of Exposure	Starting Date	Walpole HCB	Walpole OCS	Chenal Ecarte HCB	Chenal Ecarte OCS
21	Jun 8	1.29 (0.36)	1.55 (0.17)	1.63 (0.70)	2.20 (0.32)
	Aug 10	2.94 (0.71)	7.29 (2.59)	1.33 (0.16) b	3.50 (0.40) b
	Aug 31	1.53 (0.74)	2.77 (0.06)	1.71 (0.24)	3.68 (0.38)
	Nov 3	1.82 (1.10)	2.18 (1.56)	0.99 (0.08)	0.70 (0.05)
Significance					*
42	Jun 8	1.29 (0.26)	3.90 (0.12)	1.47 (0.45)	3.06 (0.58)
	Jul 20	1.17 (0.34)	3.87 (0.37)	1.55 (0.41)	4.47 (0.71)
	Aug 31	0.88 (0.07)	3.10 (0.29)	1.06 (0.09)	3.69 (0.58)
	Oct 13	1.12 (0.10)	1.48 (0.07)	1.52 (0.39)	1.40 (0.16)
Significance			***		
63	Jun 8	1.78 a	5.93 a	1.91 (0.06)	6.20 (0.32)
	Jun 29	1.13 (0.08) b	5.61 (0.85) b	1.02 (0.21)	5.77 (0.64)
	Aug 31	1.16 (0.02)	4.08 (0.12)	1.47 (0.06)	5.19 (0.23)
	Sep 21	1.30 (0.12)	2.87 (0.48)	2.35 (0.44)	3.30 (0.14)
Significance		*	*	*	***
84	Jun 8	1.12 (0.06)	4.91 (0.42)	1.05 (0.14)	5.76 (1.04)
	Aug 31	1.48 (0.04) b	4.07 (0.63) b	1.82 (0.23)	4.78 (0.65)
significance		*		*	

a, n = 1
b, n = 2
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table 4.3

Comparison of mean body burdens (1 S.E) in mussels deployed for similar exposure periods in 1986 and 1987.

		Concentration (ng/g, wet weight)			
Start Date	Exposure (weeks)	Chenal Ecarte HCB	Chenal Ecarte OCS	Walpole HCB	Walpole OCS
Jul 11/86	3	2.0 (0.34)	3.1 (0.55)	2.5 (0.17)	4.2 (0.47)
Jun 8/87		1.3 (0.36)	1.6 (0.17)	1.6 (0.70)	2.0 (0.32)
Sep 12/86	3	3.2 (0.29)	7.4 (1.60)	3.6 (0.45)	9.9 (1.92)
Aug 31/87		1.5 (0.07)	2.8 (0.06)	1.7 (0.24)	3.9 (0.98)
Jul 11/86	6	1.8 (0.29)	6.6 (1.19)	1.5 (0.33)	7.5 (1.04)
Jul 20/87		1.2 (0.34)	3.9 (0.37)	1.6 (0.41)	4.5 (0.71)
Aug 21/86	6	4.0 (0.21)	15.6 (0.51)	4.0 (0.19)	1 4 . 9 (0.86)
Aug 31/87		0.9 (0.07)	3.1 (0.29)	1.1 (0.09)	3.7 (0.58)
Jun 29/86	9	3.8 (0.24)	15.7 (0.98)	5.2 (0.58)	1 6 . 8 (1.32)
Jul 31/87		1.1 (0.08)	5.6 (0.85)	1.0 (0.21)	5.8 (0.64)

and OCS levels showed significant differences among similar exposure periods starting at different dates, there was no trend between contaminant burden and time of year (Table 4.2).

One way ANOVAs showed significant temporal heterogeneity for both HCB and OCS among mussels deployed at each of the three sites for twenty-four consecutive one week exposures periods in 1987. Although there was no seasonal shift in body burdens as in 1986, means levels of OCS accumulated after one week at the Walpole and Chenal Ecarte sites rose during July and August and then decreased to former levels in the autumn (Figure 4.2). Mean coefficients of variation (combined HCB and OCS) were similar among the sites (Walpole 29.8, Chenal Ecarte 30.6, Marsh 36.8). At the Marsh site, there was evidence that contaminant levels were most variable late in the year.

Comparison of Contaminant Accumulation Between 1986 and 1987

Among mussels deployed for similar exposure periods (starting dates within thirty days) at the same site in 1986 and 1987, body burdens were consistently lower in 1987 (Table 4.3). On average, HCB and OCS levels in 1987 were only 49 and 41 percent, respectively, of those in 1986.

Discussion

Body burdens of QCB, HCB, and OCS did not differ significantly among mussels placed in contact with the sediments or at different depths in the water. The similarity of contaminant levels in mussels suspended near the surface and those near the bottom suggests that the vertical distribution of contaminants in the water column of the lower St. Clair River at these locations is

uniform. Moreover, the similarity of concentrations in mussels in contact with the sediment suggests that body burdens are acquired from the aqueous phase rather than sediments. This latter result is concordant with prior studies on Lake St. Clair mussels which showed that their body burdens were only weakly correlated with levels in the sediment (Pugsley et al. 1985). Similarly, Muncaster et al (1988) found no significant difference in contaminant levels between mussels living in uncontaminated sand as opposed to contaminated sediments. As a consequence of the results, mussels were deployed only near the water's surface in 1987.

There have been considerable differences described for the time required for organic contaminant levels in biota to reach equilibrium with concentrations in the water. Kauss et al. (1981) found the *Elliptio complanata* accumulated detectable levels of PCBs after two to four days of exposure in the Niagara River but maximum concentrations were not reached until after 8 to 16 days of exposure. The calculated time to reach 95% equilibrium was 34 days (Kauss and Angelow, 1988). When exposed to contaminated soil in the laboratory, body burdens of PCBs in *Mytilus edulis* reached a steady state in 20 days (Pruell et at. 1986). As described earlier in this report, rates of chemical accumulation in organisms is dependent on both chemical properties (such as K_{ow}) and biological characteristics (size, respiration rate, filtering rates etc). DeKock (1983), Oliver (1984) and Defoe *et al* have provided strong evidence of the biological and chemical dependence of chemical uptake and the level of chemical equilibrium. Unfortunately, for the 1986 and 1987 studies, water concentrations were not measured, thus it is not possible at this time to provide a field verification of the previously reported lab work.

The present study showed that at exposure periods of less than

63 days, the body burdens of OCS in the mussels did not reach an equilibrium with the levels of contaminants in the water, whereas QCB and HCB are more likely to have reached equilibrium. The difference in time frame over which chemicals are integrated is a function of K_{ow}, with a lower K_{ow} corresponding to a shorter response time. The lower molecular weights of QCB and HCB may permit them to cross cell membranes more readily than OCS and thus allow a quicker equilibration with changes in the surrounding medium (Oliver 1984). Regardless, the heterogeneity in patterns of contaminant accumulation among these compounds indicates that commonly used exposure times such as three weeks are too short to achieve equilibrium for chemicals with relatively high octanol-water coefficients. The results make it clear that a single exposure time is inadequate to determine the accumulation patterns of compounds with a broad range of K_{ow}s (Hawker and Connell 1985, Konemann and van Leeuwen 1980), without having previously calibrated the biomonitor. As discussed earlier, however, when an organism has been fully calibrated, then predictions of time to equilibrium and concentration at equilibrium can be made. It is clear that future research must be focused on quantitative calibration as opposed to determining uptake patterns.

The similarity among body burdens at the Walpole, Chenal Ecarte, and Wallaceburg sites suggests that a substantial portion of the St. Clair River contaminant plume (Kauss and Hamdy 1985) enters Chenal Ecarte. As Chenal Ecarte ultimately flows into eastern Lake St. Clair, this explains the higher OCS levels previously noted in native mussels east of the central contaminant plume in Lake St. Clair (Pugsley et at. 1985). Much lower levels were observed at the Marsh site, suggesting that bioavailable contaminants are removed as the river water passes through the marsh area, perhaps as a result of sorption by vegetation or

increased sedimentation of suspended particles (Mallhot 1987, Metcalf et al. 1973).

In 1986, mussels which were deployed later in the season had consistently greater body burdens than specimens exposed for similar time periods earlier in the year. However, this pattern was not observed in either the 1987 study of consecutive one week exposures or the autumn repetition of three to twelve week exposure periods. This result suggests that greater body burdens later in the year in 1986 were the result of increased contaminant exposures.

Sequential one week exposures revealed significant short-term variation in contaminant uptake. There is a similar pattern of contaminant uptake and loss for both compounds, despite the different time it would take for the two compounds to reach equilibrium conditions. A common source within similar discharge patterns of OCS and HCB might be the reason for this similarity.

Purging studies in the field have shown that HCB is lost more quickly than OCS from mussels. Within four days of transfer to an uncontaminated site, 68 and 26 percent of the initial body burden of HCB and OCS, respectively, were purged from naturally contaminated *Lampsilis radiata* (Muncaster 1987). The differences in depuration observed in the field, (k_2 = 0.73 d^{-1} for HCB) and the laboratory (k_2 = 0.18 d^{-1} HCB) are difficult to explain. Possible changes in filtering are suspected but this needs to be verified in future calibration studies.

The body burdens of QCB and HCB observed in this study were less than those reported by Kauss and Hamdy (1985) in *E. complanata* deployed in 1982 for three weeks in the St. Clair River immediately

north of the Walpole site. Mussels deployed for 20 - 21 days at the Walpole site in this study accumulated mean QCB and HCB levels averaging 0.4 and 2.2 ng/g, respectively versus mean values of 1 and 4 ng/g for QCB and HCB in 1982. Similarly, the mean OCS level of 29 ng/g observed in 1982 by Kauss and Hamdy (1985) was much greater than the 5.2 and 3.4 ng/g averages noted in 1986 and 1987 for similar exposure periods.

The decline of 49 and 41 percent in the body burdens of HCB and OCS respectively in mussels deployed in 1987 relative to those exposed in 1986 suggests that the inputs of these compounds into the St. Clair River are declining. This observation supports data presented in the Ministry of the Environment St. Clair River MISA Pilot Site Study which indicated reduced loadings of these specific substances. Although the effect of sediment as a prolonged source of contaminants may delay further exposure reduction, the lower body burdens observed in this study may be one of the first tangible results of the reported decrease in contaminant discharge by industry along the Upper St. Clair River (Ministry of the Environment, 1988 unpublished).

CHAPTER FIVE

BIOMONITORING USING THE MAYFLY, *HEXAGENIA LIMBATA*: TOXICOKINETICS

Introduction

The resolution of the trophodynamics of contaminants in aquatic ecosystems requires detailed information about the factors which regulate organic contaminant levels and distribution in the different species of which the system is comprised. One of the major concerns in the Great Lakes is the transfer of 'in place' pollutants to species of commercial or social importance (eg. sport fish) to man. Little is known, however, if benthic biota represent an important route of chemical transfer to these species. Because there is limited information available on how benthic biota track contaminants at the sediment water interface, benthic species have been of limited use as biomonitors. The aim of this component of the MOE sponsored research program was to determine the uptake and depuration of organic contaminants in nymphs of the burrowing mayfly (*Hexagenia limbata*). The mayfly was selected as a test organism because of its importance in food web dynamics of the Great Lakes, and because of its important role in representing contaminant dynamics at the sediment water interface.

Specifically, this research addresses:

1)contaminant uptake from water, (via the gills)
2)contaminant uptake from sediment by ingestion of particulate matter
3)contaminant elimination to water, (via the gills)
4)contaminant elimination by faecal excretion

In order to assess the importance of these processes of chemical uptake and elimination, the following model was used:

$$dC_A/dt = k_1.C_w - k_2.C_A + k_s.C_s - k_e.C_A - k_R.C_A \quad (1)$$

where C_A is the chemical concentration in the mayfly, k_1 and k_s are uptake rate constants from water and sediment respectively, and k_2 and k_e are elimination rate constants to water (via gills) and to the sediments (via the GI-tract). Therefore, laboratory experiments were designed to estimate k_1, k_2, k_s and k_e. Possible seasonal variability of these contaminants was considered by measuring toxicokinetics of natural populations through the summer to late autumn period.

Methods

Chemical Uptake from Water (k_1)

Uptake from water was studied by using Whatman GC filters to remove particulates from Lake St. Clair water. The water was aerated overnight, and spiked with radio labelled HCB (10^6 mCi/mmol), using acetone as a carrier. The spiked solution was stirred with a glass rod for five minutes, and permitted to equilibrate for one hour. Five hundred ml aliquots were added to five, 1,000 ml glass beakers, one to serve as a control (no animals added) with four replicates. In order to compensate for lack of sediments to produce burrows, mayflies were provided with artificial burrows consisting of porous stainless steel mesh (5 mm 1.0 x 4 cm length).

At time zero, triplicate 2 ml water samples were removed, and placed in 12 ml of scintillation fluor. Twelve organisms were then

introduced to each beaker. After this, 2 ml water samples were collected at specified time intervals and animals retrieved. Animals were rinsed, blotted dry, weighed and transferred to vials of 12 ml scintillation fluor and allowed to extract for 24 hours (Landrum, 1988). Organisms were not digested, as this was not required for enhancing extraction efficiencies (Landrum, 1988).

Experiments were repeated during July, August, September and January 1987-88. Experiments were also run at varying water concentrations to test the assumption that k_1 is independent of water concentration.

Uptake from Sediments (k_s)

Sediments from Lake St. Clair were air dried, and a slurry of 500 mg sediment: 1500 ml distilled water was made. After addition of ^{14}C-labelled HCB, the resultant mixture was stirred overnight. After stirring, the mixture was allowed to settle, the overlying water removed, and the remaining sediment transferred to 30 ml glass beakers. The beakers were placed into a one gallon aquarium with lake water, and the entire system was permitted to settle for one day.

Six beakers were placed into three replicate aquaria, and animals were added. If a nymph did not readily establish a burrow, it was replaced with a new animal. Animals were removed, rinsed, blotted dry and uptake measured at selected time intervals. Sediment and interstitial water were measured by collecting duplicate sediment samples. Sediment samples were divided, one portion was soxhlet extracted for an estimate of total sediment contaminant load, and the second portion was centrifuged, the supernatant (pore water) collected and analyzed. The above uptake

studies were conducted for both fine and coarse sediment.

Elimination to Water and Sediments

The nature of the sediment-water requirements of the mayfly makes it impossible to study chemical elimination only to the sediment. Therefore, elimination was studied in two systems 1) to water alone (k_2) and 2) to water and sediment ($k_2 + k_E$).

To determine depuration rates, organisms were exposed to labelled HCB for twelve hours, then placed into aquaria in artificial burrows as described previously. Because of the long time period required to study elimination, animals were fed algae twice a week. A second set of animals were exposed to HCB and then placed into 30 ml beakers holding sediment and water. In both sets of experiments, 3 to 4 animals were removed at designated time intervals, and the decrease of HCB determined using liquid scintillation techniques described earlier. Experiments with sediment were also set up to determine the influence of sediment grain size on depuration kinetics.

Results

Uptake from Water (k_1)

Estimates of k_1 are summarized in Table 5.1. k_1 for HCB ranged between 20 ml g^{-1} h^{-1} in January to 95 ml g^{-1} h^{-1} in September. It is apparent in Table 5.1 that during September k_1 ranged between 92 and 95 ml g^{-1} h^{-1} in different test systems with water concentrations ranging from 0.3 to 3.5 ug L^{-1} of HCB. Figure 5.1 illustrates a typical uptake curve for HCB and also presents the associated decline of HCB in the water phase. The apparent equilibrium

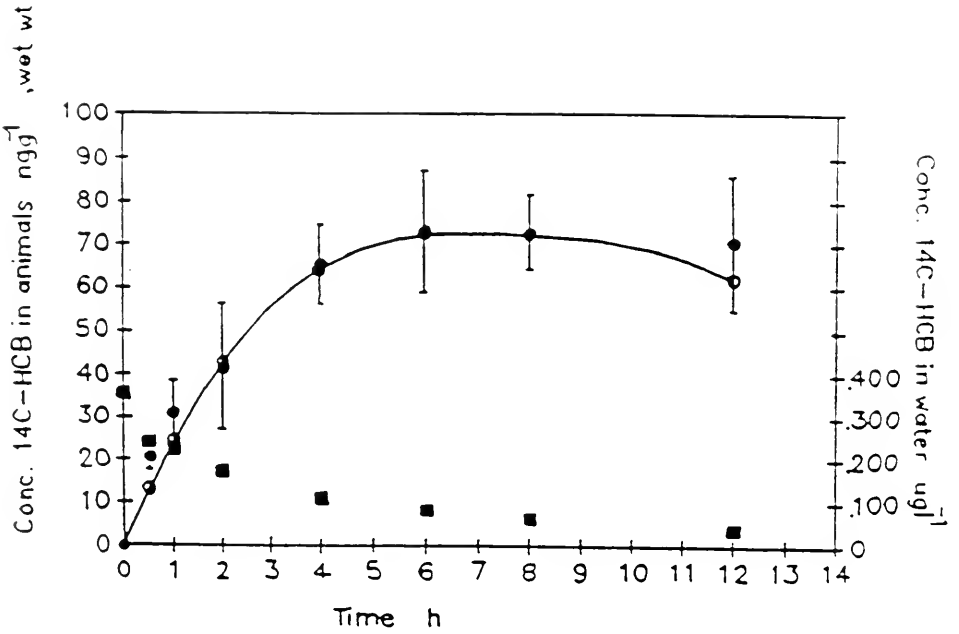


Figure 5.1

Uptake of HCB by the mayfly (*Hexagenia limbata*) from water. The closed circles represent measured values (± 1 S.D) of the concentration of 14C-HCB in the mayflies (ng/g wet weight). The open circles and solid line represent the model fit. The closed squares are the water concentrations (ug/L).

Table 5.1

Uptake rate constants (k_1) ($ml.g^{-1}.h^{-1}$) of HCB from water in mayfly populations *Hexagenia lambata*. (1987 - 1988).

Date	k_1	Initial Water Concentration (ug/L)
Jul 17	80	0.36
Jul 28	75	0.46
Aug 25	45	0.42
Sep 10	91	1.70
Sep 12	95	0.29
Sep 14	95	3.47
Jan 28	20	2.94

observed in Figure 5.1 might well be an effect of the declining concentrations of HCB in water.

As predicted, k_1 is independent of water concentrations, but evidence is provided in Table 5.1 to indicate that k_1 might vary seasonally according to the physiological condition and activity of the organism.

Uptake from Sediment (k_s)

k_s determined in the laboratory for HCB with mayfly nymphs was observed to be 0.081 g g^{-1} h^{-1} for fine sediments and 0.138 g g^{-1} h^{-1} for coarse sediments (see Table 5.2). These different estimates possibly reflected the bioavailability of HCB in sediments of different texture, with fine sediments being associated with slower transfer kinetics. In essence, the rate uptake being measured might be associated with both chemical release from the sediment to the water as well as the direct uptake from the sediment.

Interstitial water concentrations were determined during the two experiments at approximately 0.06 ug L^{-1}. Thus the different accumulation rates observed would suggest organisms were tracking sediment-bound contaminant, and not just the aqueous phase (Landrum et al. 1988). Apparently, mayflies might be capable of stripping contaminants off the particulate matter with varying efficiency dependent on the grain size of the sediments. This observation requires further study before a more definitive statement can be made.

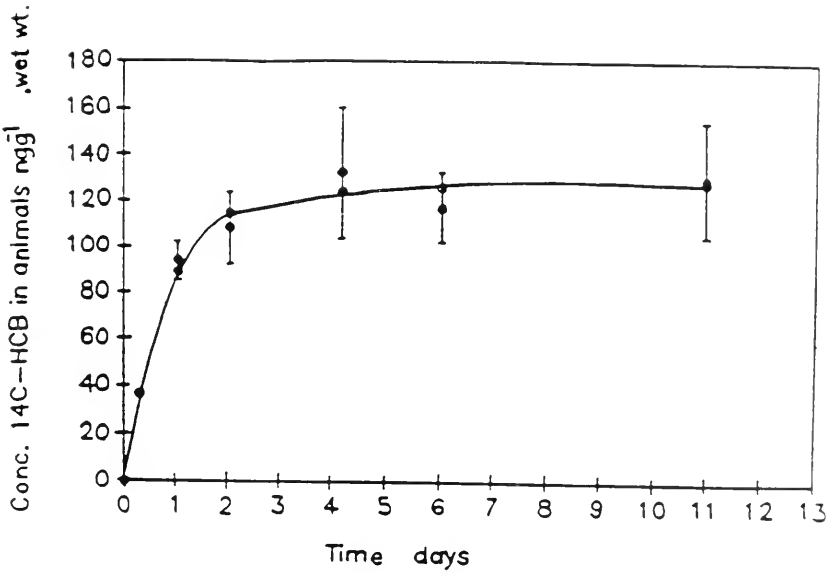


Figure 5.2A

Uptake of HCB by the mayfly (*Hexagenia limbata*) from spiked sediments (C_s is 74.9 ug/kg). The closed circles represent measured values (± 1 S.D) of the concentration of 14C-HCB in the mayflies (ng/g wet weight). The open circles and solid line represent the model fit.

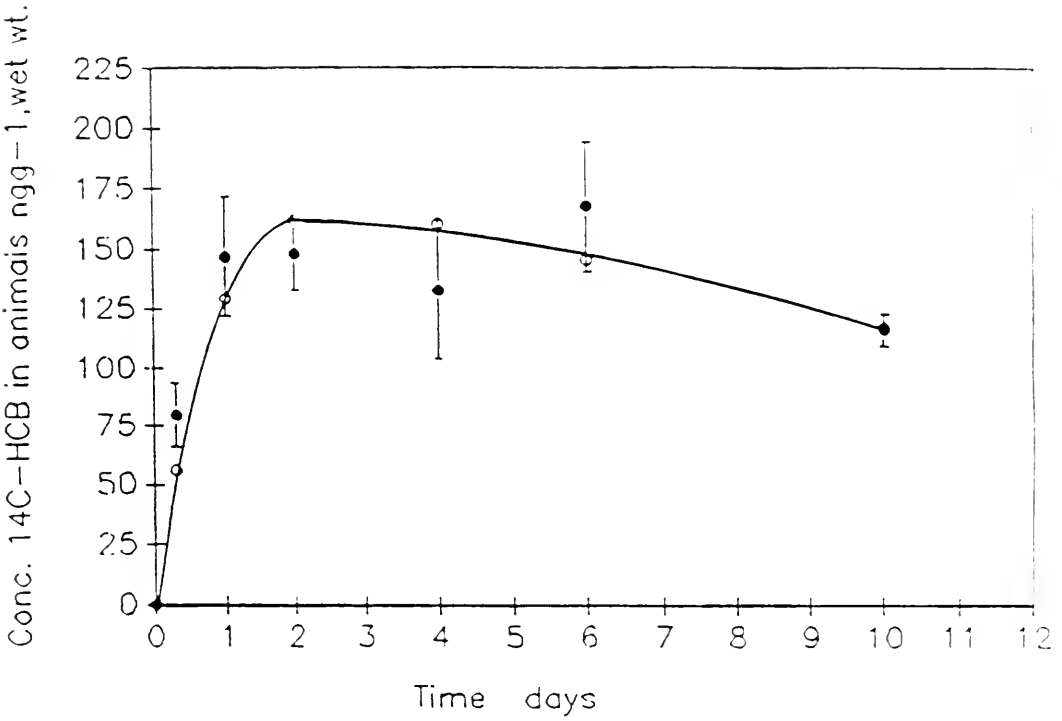


Figure 5.2B

Uptake of HCB by the mayfly (*Hexagenia limbata*) from spiked sediments (C_s is 69.0 ug/kg). The closed circles represent measured values (± 1 S.D) of the concentration of 14C-HCB in the mayflies(ng/g wet weight). The open circles and solid line represent the model fit.

Table 5.2

Uptake rate constants (k_s) ($g.g\text{-}1.h^{-1}$), sediment concentrations C_s (ug/kg dry weight) and water concentration C_w (ug/L).

Date	Sediment Type	ks	C_s	Interstitial C_w
Jan 03	Fine	0.081 ± 0.008	74.9 ± 2.8	0.057 ± 0.06
Jan 08	Coarse	0.138 ± 0.014	68.9 ± 0.8	0.062 ± 0.003

Figure 5.2A represents the dynamics of HCB accumulation from fine sediment and Figure 5.2B illustrates uptake from coarse sediments. Although the concentration of HCB was similar in the two matrices, the mayfly reached a higher body burden in the coarse sediments. Reasons for this accumulation pattern are discussed in the field section of this report.

Elimination to Water and Sediment

Depuration to water was studied seasonally, and results are summarized in Table 5.3. Values of ks for HCB in mayflies ranged from 0.026 to 0.077 h^{-1}, and were not related to the chemical concentrations in the organism. Also presented in Table 5.3 are estimates of the chemical's half live $t_{0.5}$ (which ranged from 9 hours to 26 hours) based on the observed depuration rates. The time required to eliminate 95% of the body burden ($t_{0.95}$) ranged from 45 to 133 hours. The mayfly had measured values of k_2 for HCB about ten times higher than the mussel *Elliptio complanata* (k_2 = 0.008 h^{-1}). This is not unexpected, and suggests how allometric relationships amongst organisms might be used in the future to select biomonitors according to the appropriate time frames required to approach steady state conditions.

Figure 5.3 illustrates typical depuration dynamics of HCB to water observed for the mayfly. It is apparent that depuration follows first order kinetics as assumed in the models used to calibrate organisms as biomonitors.

Figure 5.4 illustrates that the depuration rates observed in systems with and without sediment did not vary significantly. As noted in Table 5.4, average values of k_2 ranged from 0.026 to 0.027 h^{-1}, and average values of $k_2 + k_E$ (with sediment) increased from

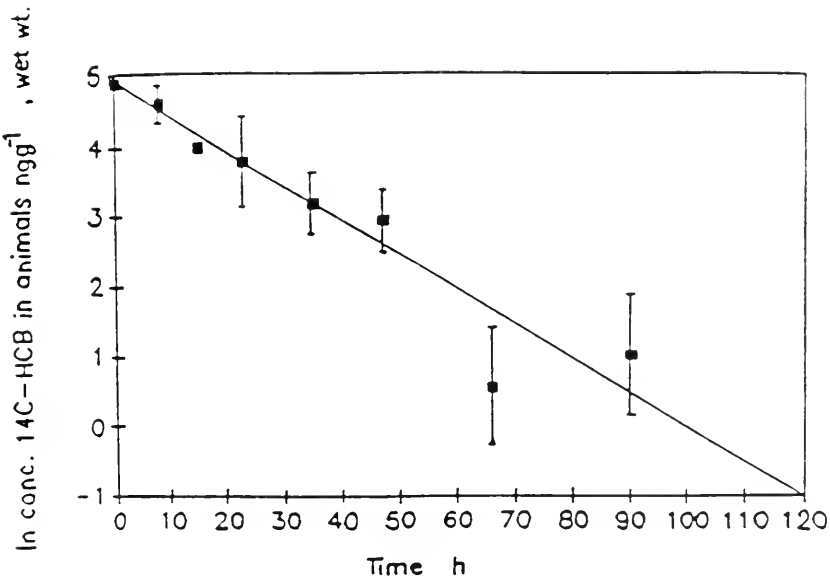


Figure 5.3

Depuration of 14C-HCB from the mayfly in water: The logarithm of the 14C-HCB concentration in the mayfly (ng/g wet weight) versus time.

Table 5.3

Depuration rate constants k_2 (hr^{-1}) of HCB in mayfly populations (*Hexagenia limbata*), the concentration C_A in the organism (mg. g^{-1}, the half life time $t_{0.5}$ (hr) and the time to achieve 95% of steady-state $t_{0.95}$ (hr)

Date	k_2	C_A*	$t_{0.5}$	$t_{0.95}$
Jul 17	0.049	92.8	14	70
Jul 28	0.046	113.3	15	75
Aug 16	0.071	36.6	10	49
Aug 16	0.077	357.2	9	45
Aug 19	0.048	132.3	14	72
Aug 22	0.062	12.1	11	56
Aug 28	0.027	247.2	25	128
Nov 9	0.026	158.1	26	133

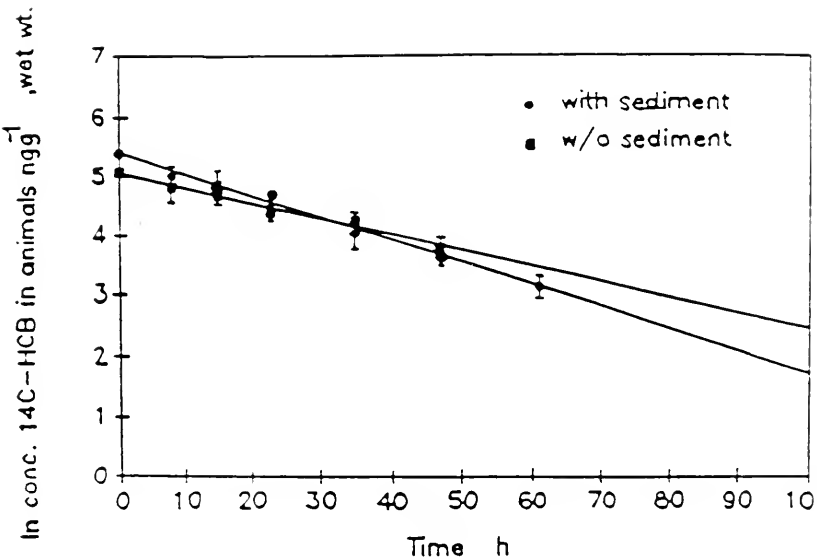


Figure 5.4

Depuration of 14C-HCB in the presence (circles) and absence (square symbols) of sediments: The logarithm of the 14C-HCB concentration in the mayfly (ng/g wet weight) versus time.

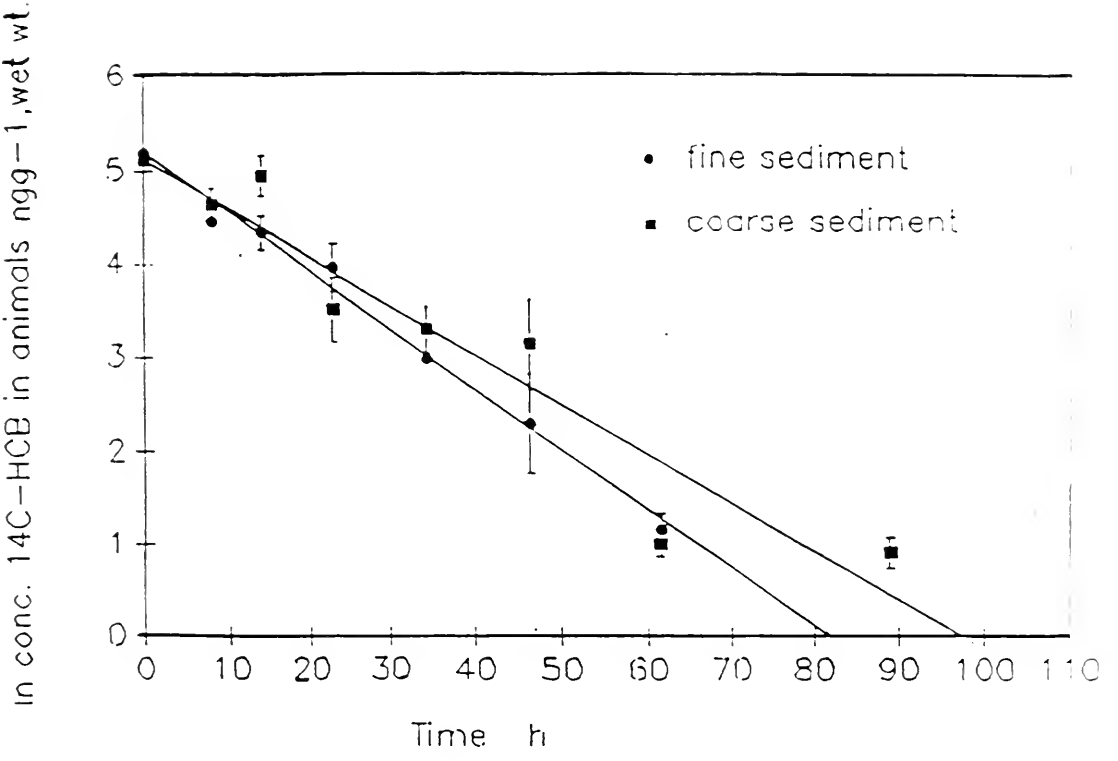


Figure 5.5

Depuration of 14C-HCB in fine (circles) and coarse (squares) sediments: The logarithm of the 14C-HCB concentration in the mayfly (ng/g wet weight) versus time.

Table 5.4

Depuration rate constants (hr^{-1}) of HCB in *Hexagenia limbata* in systems with and without sediments and in systems of coarse and fine sediments at different concentrations in the organism C_A (ug/kg wet weight). r^2 is the correlation coefficient of the linear regression of log C_A versus time.

Substrate	k_2	C_A	r^2
Sediment ($k_2 + k_e$)	0.034 ± 0.008	143	0.65
No Sediment (k_2)	0.027 ± 0.004	247	0.72
Sediment ($k_2 + k_e$)	0.037 ± 0.005	220	0.78
No Sediment (k_2)	0.026 ± 0.009	158	0.53
Fine Sediment ($k_2 + k_e$)	0.052 ± 0.008	167	0.82
Coarse Sediment (k_2)	0.063 ± 0.010	177	0.89

0.034 to 0.037 h^{-1}. There was no significant difference in the rate constants suggesting that k_e is relatively small compared to k_2 (i.e. Contaminant loss in mainly to the water and not directly to the sediment).

Further observations on the possible influence of sediment on depuration kinetics, were made in systems with coarse and fine sediments and results are summarized in Figure 5.5 and Table 5.4. There was no significant difference in chemical elimination ($P > 0.10$) in the two systems, again supporting the observation that k_e does not play an important role in chemical accumulation and elimination in mayflies.

Discussion

Observed levels of chlorinated hydrocarbons in field populations of the mayfly (*Hexagenia limbata*) and in associated sediments are illustrated in Figure 5.6, page 61. Data presented in Figure 5.6 are based on contaminant levels per g lipid and per g organic carbon (measured as loss on ignition). For lower k_{ow} compounds such as QCB, HCB and PCB 101, levels in the mayfly are below those calculated assuming an equilibrium would be established between the sediments and the organisms (see the section on field observations). The lower levels of compounds such as QCB and HCB might reflect that different contaminant sources other than the sediments were being tracked by the Mayfly nymphs.

Of considerable interest in this study is that although sediments have small effects on depuration rates, they do influence chemical uptake. Uptake from the sediment, studied by using radio-labelled sediments, does not provide, however, a verification that uptake was directly from the sediments via transfer within the gut.

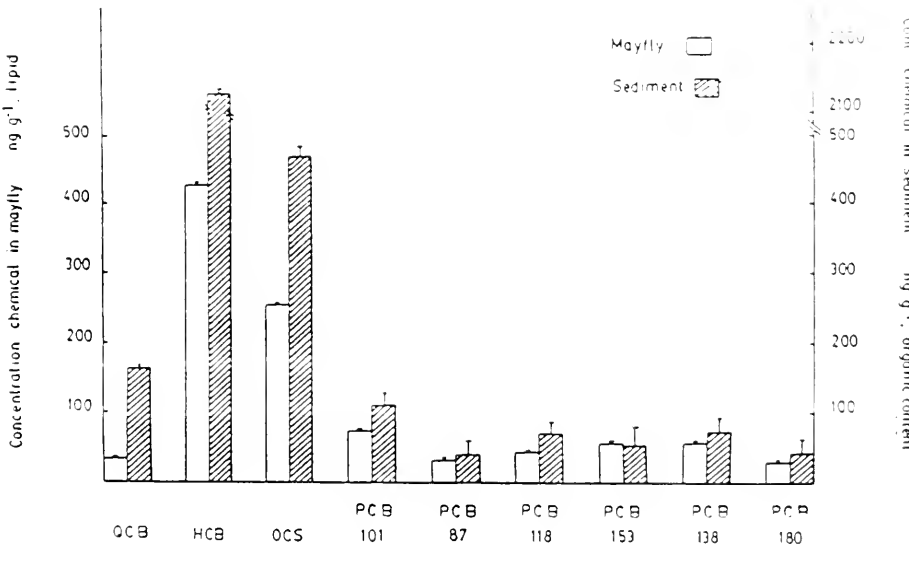


Figure 5.6

Concentrations of selected chlorinated hydrocarbons in mayfly tissue and bottom sediment, collected at Site 8 in July-Sept. 1987.

Another possible mechanism would be dissolution of the contaminant from the sediment followed by incorporation into the mayfly. If the rate of dissolution from the sediment was much lower than the rate of uptake, then water concentrations would 'appear' to be near zero, even though the water acts as the regulating transfer medium. Landrum et al (1989) concluded sediments were the major source of contaminant uptake, but both the field observations and the kinetic data presented here would not rule out the possible role of water exposure. For higher k_{ow} compounds, however, because of their affinity for the sediment, water might not be an important transfer mechanism.

The following section of field observations (Chapter 5) discusses two possible models of contaminant accumulation in mayfly populations, and the achievement of equilibrium concentrations. Although sediment might be an important source of high k_{ow} compounds, there needs to be further verification of the role of sediments in the uptake of low (log k_{ow} < 6.0) k_{ow} compounds.

The use of the model presented in equation 1 gives a good estimate of the processes regulating contaminant levels in mayfly nymphs. It is essential, however, that future work resolve the role of water mediated chemical transfers between the sediments and the organism. The greater the role of water as a mediator, the less likely it will be that organisms will be in chemical equilibrium with their surrounding sediments. Mixing of pelagic water with pore water might move the chemical burden of an organism away form the expected steady state relative to contaminant concentrations in the sediment.

BIOACCUMULATION OF CHLORINATED HYDROCARBONS BY THE MAYFLY (*HEXAGENIA LIMBATA*) IN LAKE ST. CLAIR

Introduction

In aquatic environments, hydrophobic organic chemicals show a tendency to partition into sediments thus reaching concentrations in sediments which are several fold higher than those in water. Benthic invertebrates, such as the burrowing mayfly nymph *Hexagenia limbata*, which dwell in the sediments and feed on sediment-associated matter, are thus exposed to relatively high concentrations of sediment-associated organic substances. As a first step in an assessment of the potential risk of sediment associated organic substances to benthic invertebrate communities, the actual exposure of the organism needs to be determined.

Various authors have addressed the uptake of organic substances in benthic invertebrates in the laboratory and in the field. For example, Landrum (1988) and Eadie et al. (1983) determined the kinetics of some aromatic hydrocarbons in the amphipod *Pontoporeia hoyi* in the laboratory and in the field. Oliver (1984, 1987) demonstrated uptake and accumulation of various organochlorines in oligochaete worms (*Limnodrilus hoffmeisteri* and *Tubifex*). Recently, Landrum and Poore (1988) reported laboratory measurements of the uptake and depuration kinetics of some aromatic hydrocarbons in the mayfly (*Hexagenia limbata*), which is a dominant benthic species in Lake St. Clair.

The objective of this study is to investigate the mechanism of organic chemical uptake and bioaccumulation in the mayfly under

typical field conditions in Lake St. Clair. The study investigates the relationship between chemical concentrations in sediments and *in-situ* mayflies for chemicals of varying hydrophobicity (1-octanol-water partition coefficient) and develops models and correlations for exposure assessment of hydrophobic organic substances in benthic invertebrates.

Theoretical

To describe the exchange of organic chemicals between sediments and mayflies, models of various complexity can be proposed. We will briefly present two models, which have been discussed before to various degrees (Pavlou and Weston 1984, Reuber et al. 1987, Landrum and Poore 1988, Gobas et al. 1988). The models will be treated as hypotheses, which will be tested experimentally in the field. This approach is believed to improve insights into the mechanism of chemical exchange between sediments and benthic invertebrates such as the mayfly.

Model I

The simplest approach towards describing chemical interactions between sediments and the mayfly is to assume that the chemical in the mayfly is in chemical equilibrium with that in the sediment. This equilibrium condition can be characterized by equal fugacities of the chemical in the sediment and in the mayfly:

$$f_s = f_B \qquad (1)$$

where f_s and f_B are the fugacities (Pa) in respectively the sediments and the organism. To test if this equilibrium condition applies in real situations requires the measurement of f_s and f_B in

the field. Unfortunately, f_s and f_B cannot be directly measured. However, Mackay and Paterson (1981, 1982) have shown that chemical fugacities in sediments and organisms can be determined from the chemical concentrations in the sediment (C_s) and the organism (C_B) if the chemical fugacity capacity of the sediments (Z_s) and that of the organism (Z_B) are known i.e.:

$$f_s = C_s/Z_s \quad (2)$$

$$f_B = C_B/Z_B \quad (3)$$

The fugacity capacities Z_s and Z_B of a chemical can be determined as:

$$Z_s = k_s.d_s/H \quad (4)$$

$$Z_B = k_B.d_B/H \quad (5)$$

where k_s is the sediment-water partition coefficient (L/kg), k_B is the organism-water partition coefficient (L/kg), d_s and d_B are the densities (kg/L) of respectively the sediment and the organism and H is the Henry Law constant ($Pa.m^3/mol$).

Karickhoff et al. (1979) and Karickhoff (1981) have demonstrated that for hydrophobic organic chemicals at low concentrations, (which are often encountered in the environment), k_s can be viewed as the product of the organic carbon fraction of the sediment (on a mass fraction basis) X_s (kg/kg) and the organic carbon partition coefficient k_{oc} i.e. k_s equals $X_s.k_{oc}$. DiToro (1985) has suggested that, within experimental error, the organic carbon partition coefficient k_{oc} equals the 1-octanol-water partition coefficient k_{ow} i.e.:

$$k_{oc} = k_{ow} \quad (6)$$

It thus follows that Z_s can be determined as:

$$Z_s = X_s.k_{ow}.d_s/H \quad (7)$$

Gobas et al. (1989) have shown that the organism-water partition coefficient k_B is controlled by the lipid content (on a mass fraction basis[1]) of the organism L_B (kg/kg) and k_{ow}. It can thus be expressed as:

$$k_B = L_B.k_{ow} \quad (8)$$

The fugacity capacity in the organism, Z_B can thus be determined as:

$$Z_B = L_B.k_{ow}.d_B/H \quad (9)$$

It thus follows from equations 2,3,7 and 9 that if there is a chemical equilibrium between sediments and mayflies (i.e. $f_s = f_B$) the ratio of chemical concentrations in the organism and the sediment should equal:

$$C_B/C_s = (L_B.k_{ow}.d_B/H)/(X_s.k_{ow}.d_s/H) = L_B.d_B/X_s.d_s \quad (10)$$

This demonstrates that at equilibrium the organism/sediment concentration ratio C_B/C_s is determined only by organism and sediment characteristics, namely L_B, d_B, X_s and d_s. The nature and properties of the chemical (e.g. k_{ow}) should not influence C_B/C_s. In other words, if the equilibrium assumption applies in real field situations C_B/C_s should be similar for all organic chemicals i.e. $L_B.d_B/X_s.d_s$.

---

[1]The density of the wet weight organism is approximately 1.0 kg/L

Model II

The equilibrium model outlined above treats the mayfly as a single homogenous phase, which is in thermodynamic equilibrium with the sediments. It ignores the actual physiological processes of chemical exchange between the organism, the sediments and other relevant compartments such as the water (i.e. pore and overlying water). The second model that we will discuss views chemical uptake and bioaccumulation as a result of a balance between the rates of chemical uptake (i.e. from water and sediments) and depuration. Chemical uptake is from the water (i.e. via the gills) and the ingestion of contaminated sediments (i.e. via the gastro-intestinal tract). Depuration is through direct chemical elimination to the water (via the gills), elimination into egested "faecal" matter (via the gastro-intestinal tract) and metabolic transformation (i.e. for metabolizable chemicals). The organism is thus defined as the whole organism excluding the contents of the gastro-intestinal tract.

The expression describing simultaneous exchange of chemical between the benthic organism and water, the organism and ingested sediment particles, and metabolic transformation, can be expressed as:

$$dC_B/dt = k_w.C_w - k_e.C_B + k_s.C_s - k_f.C_B - k_M.C_B \qquad (11)$$

where C_B, C_w and C_s are the chemical concentrations (mol/m^3) in respectively the organism, the water and the sediments and t is time (h). The first order rate constants k_w, k_e, k_s, k_f and k_M refer to respectively chemical uptake from the water (via the gills), elimination to the water (via the gills), uptake from ingested sediment associated matter (via gastro-intestinal tract),

elimination in egested matter (via the gastro-intestinal tract) and metabolic transformation.

Under field conditions, where organisms have been continuously exposed to approximately constant chemical concentrations in the water and sediments, it seems reasonable to assume that the chemical concentration in the organism is in steady-state with that of the sediments and the water in its immediate environment. In other words, there is no net uptake in, or loss of chemical from the organism i.e. dC_B/dt is zero. Equation 11 can thus be simplified to:

$$C_B = (k_w.C_w + k_s.C_s)/(k_e + k_r + k_M) \qquad (12)$$

Rearranging equation 12 gives the following expression for the organism/sediment concentration ratio C_B/C_s:

$$C_B/C_s = (k_w.(C_w/C_s) + k_s)/(k_e + k_r + k_M) = ((k_w.C_w/C_s) + k_s)/k_t \qquad (13)$$

where k_t is the total depuration rate constant i.e $(k_e + k_r + k_M)$. Equation 13 demonstrates that the relationship between organism and sediment concentrations (i.e. C_B/C_s) is predominantly the result of a balance between uptake and elimination rate constants. It thus reflects organism and chemical dynamics.

Methods

Mayfly and Sediment Collection

One-year old mayfly nymphs (*Hexagenia limbata*) and sediment samples were collected simultaneously with a Peterson grab from a specific site in Lake St. Clair (Latitude N : 42.30'.00'', Longitude W : 82.42'.25'') in July, August and September of 1987.

This site was chosen because it maintained a continuous population of nymphs throughout the season.

Mayflies were separated from the substrate with a 625 um mesh sieving bucket (in the field) and held with the sediment and overlying lake water for transport. In the laboratory, nymphs were separated from the sediment with a 250 um mesh sieve, wrapped in hexane rinsed aluminum foil and stored at -20°C until further analysis. Samples were processed within 3 to 4 hours after collection to minimize contaminant losses. Sediment samples were stored in amber glass jars at -20°C until analysis.

Mayfly Analysis

Extraction and contaminant analysis were performed as described previously by Pugsley et al. (1985) and Ciborowski and Corkum (1988). Briefly, individual mayflies were homogenized in 120 mL of acetonitrile and 40 mL water for 1.5 min. with a polytron blender. The homogenate was filtered under vacuum through a sintered glass filter. The residue on the filter was re-suspended in 50 mL acetonitrile and then filtered. This procedure was repeated twice again with 20 mL acetonitrile. After adding 1 mL of concentrated sulphuric acid the combined filtrate was extracted, respectively once with 150 mL and twice with 75 mL petroleum ether. The combined extract was washed with 200 mL distilled water. To remove dissolved water the extract was passed through a 15 g anhydrous sodium sulphate column. Clean-up of the extracts was performed on 0.40 x 0.02 (I.D.) m glass columns containing 0.02 m anhydrous sodium sulphate and 20 g of Florisil. Columns were eluted with approximately 200 mL petroleum ether. After clean-up, extracts were concentrated by evaporation to 10 mL, and then analyzed by gas chromatography. Because of experimental

difficulties with spiking mayflies, recovery efficiencies of the test chemicals were determined using mussel tissue and ranged from 75 to 85%. The lipid content of the mayflies was determined by evaporating all solvent from dried mayfly extracts, after which the lipids were determined by weight.

Sediment Analysis

Of each sediment sample two 5 g sediment sub-samples were oven-dried at 106°C, then weighed and extracted individually by soxhlet-extraction with 300 mL 1:1 acetone:hexane (v/v) for 16 hours. The extract was concentrated by evaporation to approximately 50 mL and passed through a 15 g anhydrous sodium sulphate column to remove water. Clean-up of the extract was similar to that used for the mayflies, but hexane was the eluting solvent. The extract was then concentrated by evaporation to approximately 5 mL and treated with activated copper (i.e. copper powder treated with a 5% nitric acid solution, then washed with distilled water, acetone and hexane). This solution was diluted to 10 mL and analyzed by GC. Recovery efficiencies of the entire sediment extraction procedure for the individual chemicals and PCB congeners were determined with spiked sediments and ranged from 79 to virtually 100%.

The organic fraction of the sediments was determined, independent of the chemical analysis, by measuring the reduction in mass of an oven-dried 5 g sediment sub-sample after overnight heating at 550°C to burn off organic matter. This method, which determines the volatile organic matter content, has been suggested as underestimating to some extent the "actual" organic carbon content of the sediment, which is more correctly measured by an organic carbon analyzer (Landrum 1989a)as TOC.

Gas Chromatographic Analysis

All samples were analyzed on a Hewlett-Packard 5790A gas chromatography equipped with a ^{63}Ni-electron capture detector at 300°C, a splitless injector at 250°C, a 25m J&W Scientific DB5 fused silica capillary column (film thickness: 0.25 um) and a Hewlett-Packard 3390A integrator. Carrier gas was ultra-high-pure-grade helium at a flow rate of 1.5 mL/min. Make up gas i.e. ultra-high pure 5% methane-argon was applied at a flow rate of 35 ml/min. The temperature program for GC analysis was : 50°C for 0.5 min., then increasing to 255°C at 3°C/min., at which the temperature remained constant for 15 min. Standards used for qualitative identification (i.e. retention times) and quantification were prepared from >98% pure commercially obtained substances and individual PCB congeners. GC-analysis was performed for pentachlorobenzene (QCB), hexachlorobenzene (HCB), Octachlorostyrene (OCS), and the polychlorinated biphenyl (PCB) congeners 87, 101, 118, 138, 153 and 180.

Statistical Analysis

Standard deviations are reported in parentheses. Confidence intervals are reported in square brackets and have a 95% probability.

Results & Discussion

Sediment and Mayfly Characteristics

The sediment at our field site was predominantly silt-clay with a density of 1.4 kg/L. The organic content of the sediment (X_s) was determined to be 3.62 (± 0.54) %. The lipid content (L_s)

of the mayflies was 2.54 (± 0.58) % when expressed on a wet weight, whole organism basis.

Bioaccumulation in the Mayfly

The observed concentrations of 6 PCB congeners, octachlorostyrene, pentachlorobenzene and hexachlorobenzene in the mayfly and in the sediments throughout the July-September period are listed in Table 5.5, page 78. During this period, the variability in the mayfly and sediment concentrations was relatively small. The mean values of the mayfly and sediment concentrations are thus believed to adequately represent the chemical concentrations during the July-September period. For each substance, the mayfly/sediment concentration ratio was determined by dividing the mean chemical concentration in the mayfly by the mean concentration in the sediments. These concentration ratios are listed in Table 5.5 and plotted versus the 1-octanol-water partition coefficient (k_{ow}) in Figure 5.7, page 77. Figure 5.7 demonstrates that C_B/C_S varies from 0.14 for pentachlorobenzene to 0.71 for PCB-153 and tends to follow a linear relationship with k_{ow} when expressed on a logarithmic basis i.e.

$$\log(C_B/C_S) = 0.37\ [\pm\ 0.09].\log k_{ow} - 2.76\ [\pm\ 0.17] \qquad (14)$$
$$n = 9,\ r^2 = 0.93$$

This correlation indicates that when chemical concentrations in sediments are the same, higher k_{ow} chemicals tend to achieve higher concentrations in the mayfly.

Table 5.5

Concentrations of pentachlorobenzene, hexachlorobenzene, octachlorostyrene and six PCB congeners in the mayfly (in ng/g wet weight) and in the sediments (in ng/g dry weight in Lake St. Clair from July to September. Deviations are in parentheses.

	Pentachloro-benzene	Hexachloro-benzene	Octachloro-styrene	PCB-101	PCB-87	PCB-118	PCB-153	PCB-138	PCB-180
July:									
Mayfly	0.50	8.8	5.4	1.7	0.70	0.87	0.94	ND	0.62
(n=4)	(0.07)	(0.8)	(1.2)	(0.4)	(0.42)	(0.59)	(0.66)		(0.31)
Sediments	6.0	73.2	17.6	2.9	1.20	2.1	1.82	2.36	0.83
(n=2)	(0.13)	(18.0)	(0.8)	(0.6)	(0.32)	(0.6)	(0.65)	(0.87)	(0.28)
August:									
Mayfly	1.2	14.0	8.2	1.7	0.7	1.1	1.3	1.22	0.94
(n=2)	(0.3)	(0.97)	(0.7)	(0.2)	(0.01)	(0.1)	(0.2)	(0.04)	(0.02)
Sediments	6.1	135.0	17.2	3.8	1.6	2.6	2.0	2.8	1.0
(n=1)									
September:									
Mayfly	1.13	11.4	6.6	2.3	1.1	1.5	2.5	1.7	1.5
(n=2)	(0.45)	(0.73)	(0.8)	(0.7)	(0.3)	(0.5)	(0.2)	(0.5)	(0.5)
Sediment	5.9	51.7	16.3	5.3	1.7	3.2	2.2	3.0	2.8
(n=2)	(0.5)	(7.4)	(1.3)	(0.5)	(0.5)	(0.6)	(0.1)	(0.2)	(0.5)
C_B/C_S	0.14	0.14	0.37	0.46	0.54	0.41	0.71	0.54	0.62
	(0.08)	(0.09)	(0.11)	(0.27)	(0.34)	(0.27)	(0.51)	(0.29)	(0.65)
LOG k_{OW}	5.03[A]	5.45[A]	6.29[B]	6.40[C]	6.50[C]	6.40[C]	6.90[C]	7.00[C]	7.00[C]

A from Miller et al. 1985

B from Veith et al. 1979

C from Shiu and Mackay 1989

Sediment-Mayfly Interaction

To obtain a better understanding of the actual mechanism of uptake and accumulation of hydrophobic organic substances in the mayfly under field conditions the experimental data in Table 5.5 were used to test the models I and II discussed earlier. This is illustrated in Tables 5.6, and 5.7, page 79.

Model I demonstrates that if there is a chemical equilibrium between the mayfly and the sediment C_B/C_S for each non-metabolized chemical should equal $L_B.d_B/X_S.d_S$. In our particular field situation this ratio is 0.025x1.0/(0.036x1.4) or 0.50 (± 0.09). Figure 5.7, page 77, and Table 5.1, page 62, illustrate that within the margins of experimental uncertainty, the observed C_B/C_S ratios for the higher k_{ow} substances (i.e. log k_{ow} > 6) in Lake St. Clair agree with the model I prediction. It should be noted that the C_B/C_S ratio predicted by model I (i.e. $L_B.d_B/X_S.d_S$) is dependant on correct values for L_B and X_S. The methodologies used to derive L_B and X_S are therefore critical. As mentioned earlier, our method for measuring X_S may somewhat underestimate the actual organic carbon content and thus overestimate $L_B.d_B/X_S.d_S$. If X_S is actually higher than our measured value of 0.036, e.g. by a factor of 2, then the model I predicted C_B/C_S ratio drops also by a factor of 2 to 0.25. In that case, all observed C_B/C_S ratios do not significantly differ from the model I predicted value. This indicates that f_S and f_B are approximately equal, thus suggesting that the mayfly and sediments are at or near chemical equilibrium.

Although the observed C_B/C_S ratios tend to correspond with the equilibrium value, they are not normally distributed around the equilibrium value. In fact, Figure 5.7 indicates a correlation between the C_B/C_S ratio and k_{ow}, which can not be readily explained

Table 5.6

Parameters for mayfly-sediment chemical equilibrium model (i.e. Model I).

Mayfly Density:	1.0 kg/L
Lipid Content:	2.54 (± 0.58) %
Sediment Density:	1.4 kg/L
Organic Content:	3.62 (± 0.54) %

Table 5.7

Parameters for the dynamic mayfly-sediment model (i.e. model II) and model calculations.

Chemical	k_s (h^{-1})	k_T [a] (h^{-1})	C_s/C_s [b] model II	C_s/C_s observed
QCB	0.049	0.027	1.8	0.14
HCB	0.049	0.023	2.1	0.14
OCS	0.049	0.015	3.3	0.37
PCB-101	0.049	0.014	3.5	0.46
PCB-87	0.049	0.013	3.8	0.54
PCB-118	0.049	0.014	3.5	0.41
PCB-153	0.049	0.009	5.5	0.71
PCB-138	0.049	0.008	6.1	0.54
PCB-180	0.049	0.008	6.1	0.62

(a) Calculated from K_{ow} (Table 5.5) according to $k_T = -0.01.\log K_{ow} + 0.08$

(b) Calculated as k_s/k_T

by equilibrium partitioning. We will therefore investigate the applicability and validity of the more "realistic" dynamic model II. This model (i.e. equation 12) considers various rate constants for chemical uptake and elimination in the mayfly, which can not be readily measured in the field. However, laboratory measurements of the uptake and depuration kinetics in the mayfly by Landrum and Poore (1988) have demonstrated that: (i) The metabolic transformation capability of the mayfly for chlorinated hydrocarbons is probably insignificant (i.e. $k_M = 0$). (ii) In laboratory experiments k_s is approximately 0.049 g dry sediment/g mayfly/h and is not dependent on k_{ow}. (iii) The depuration rate constant (i.e. k_T in h^{-1}) tends to drop with increasing k_{ow} (i.e. $k_T = -0.01.\log k_{ow} + 0.08$). In addition, the authors estimated that under typical field conditions chemical uptake in the mayfly from water is insignificant relative to uptake from sediment. This suggests that the $k_w.C_w$-term in equations 11 and 12 may be insignificant under field conditions, such that model II (i.e. equation 13) can be simplified to:

$$C_B/C_s = k_s/k_T = k_s/(k_E+k_F) \qquad (15)$$

Based on the laboratory measurements of k_s (i.e. 0.049 g/g/h), k_T (i.e. $k_T = -0.01.\log k_{ow} + 0.08$) and k_{ow}, equation 15 can be used to predict the C_B/C_s ratios in the field. Figure 5.7, page 77, and Table 5.7, page 79, illustrate the observed and predicted values.

Figure 5.7 demonstrates that the combination of equation 15 and the laboratory kinetic data by Landrum and Poore (1988) correctly predicts the k_{ow} dependence of C_B/C_s i.e. the slopes of the correlations between predicted and observed log C_B/C_s values and log k_{ow} are approximately similar. This suggests that the increase in the observed C_B/C_s values with k_{ow} may be due to a drop in the total

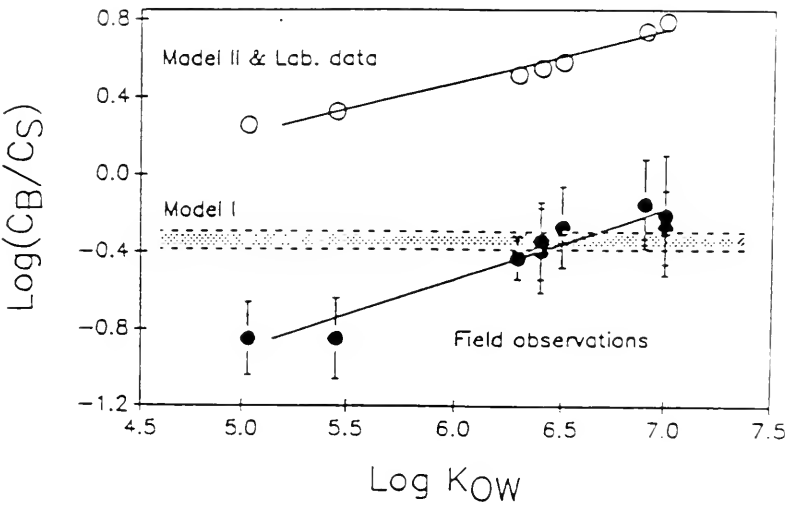


Figure 5.7

Logarithms of observed mayfly/sediment concentration ratios of selected organochlorines in Lake St. Clair (closed circles) with their 95% confidence intervals versus log k_{ow}. The shaded area represents predictions of a sediment-organism chemical equilibrium model (i.e. model I). The open circles are predicted values from a dynamic sediment-mayfly model (i.e. model II), calibrated by laboratory data from Landrum and Poore (1989).

depuration rate constant when k_{ow} increases. This drop in the depuration constant is possibly due to the lower tendency of higher k_{ow} chemicals to "partition" from the mayfly to the water. This indicates that chemical uptake in the mayfly, and thus C_B/C_s, is the result of a dynamic process in which chemicals are taken up from the sediment at a constant rate and are eliminated to the water (k_E) and to egested "faecal" phases (k_F). The lower k_{ow} chemicals, which have higher depuration rate constants to the water, thus have a lower C_B/C_s. But when k_{ow} increases and the depuration rate constants to the water (k_E) drops, chemical elimination to the water becomes a less significant depuration route, thus allowing the mayfly and sediment to approach chemical equilibrium or k_s/k_F. It should be stressed, however, that this interpretation of model II is only valid if the water is an insignificant source of chemical uptake in the mayfly. This may correspond with a situation in which water and sediments are not in chemical equilibrium, but f_s exceeds f_w.

The large difference in C_B/C_s ratios between model/laboratory predictions and field observations, which is approximately a factor of 10, is puzzling and may reflect either experimental error or a real laboratory-field difference. To explain this large difference between laboratory and field observations Landrum (1989b) has suggested that as a result of a longer contact-time between chemical and sediment in the field, sediment-associated chemicals in the field are less "extractable" (or bioavailable) by organisms than those in laboratory experiments which generally have had a short contact time. The same phenomenon may also affect the ratio of chemical concentrations in ingested sediments and gill extracted water. Due to a longer sediment-chemical contact-time in the field the C_w/C_s ratio in the field may be lower than that in laboratory experiments. This could results in a more important contribution

of chemical uptake from the water (i.e. the $k_1.C_w$-term in equation 11) to the total body burden of the mayfly in the laboratory than in the field. Further research is required to resolve this issue.

Conclusion

It can be concluded that at our field location in Lake St. Clair organochlorine concentrations in mayflies and sediments tend to be at or near chemical equilibrium. This study suggests that at a first approximation the relationship between mayfly and sediment concentrations can be expressed by a simple equation, i.e. $C_B = C_S.L_B.d_B/X_S.d_S$, which only requires information of the lipid content of the mayfly, the organic carbon fraction of the sediments and the densities of the sediment and the mayfly at a particular site. However, a constant C_B/C_S ratio for all chemicals, such as predicted by the chemical equilibrium model, has not been observed. The C_B/C_S ratio tends to increase with increasing k_{ow}. A dynamic model, which treats uptake and bioaccumulation in the mayfly as a balance of the rates of chemical uptake from water and sediments and depuration to water and other (e.g. "faecal") phases, may thus give a more realistic and accurate description of organochlorine exposure to mayflies. However, when calibrated with rate constants derived from laboratory experiments this model correctly predicts the k_{ow}-dependence of the C_B/C_S ratio but overestimates the actual C_B/C_S ratios by approximately a factor of 10. This indicates that at this stage observation of chemical bioaccumulation in the field and in the laboratory are inconsistent and that further research is required to enable reliable predictions of chemical concentration in mayflies in the field. This work is essential in order for the Ministry of the Environment to interpret the ecological impact of their in-place pollutant studies.

CHAPTER SIX

BIOMONITORING WITH *MYRIOPHYLLUM SPICATUM* : KINETICS OF CHEMICAL UPTAKE, DEPURATION AND BIOCONCENTRATION OF ORGANOCHLORINES

Introduction

Because of their lack of mobility and their abundance in many aquatic systems, aquatic macrophytes species have the potential to function as *in-situ* biomonitors of waterborne contaminants. For example, in the St. Clair River, levels of HCB and OCS in aquatic macrophytes such as *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Najes flexillis* were in the order of 0.3 - 1.6 and 0.4 - 0.74 ug/kg respectively (GLI - MOE second annual report). The use of *in-situ* aquatic plants as biomonitors is especially attractive since they may require only a minimum of work on the part of the user. The plants only need to be collected and analyzed.

However, before aquatic macrophytes can be applied as biomonitors of environmental contamination, some important issues need to be addressed, namely:

(1) To what extent are aquatic macrophytes able to absorb contaminants from the ambient water
(2) What are the rates of chemical uptake directly from the water, and through the roots, and what are the rates of chemical depuration.
(3) How long do aquatic plants need to be exposed before the contaminant concentration in the plant can be simply related

to the ambient water concentration.

(4) What are the analytical techniques that should be applied to determine the chemical concentration in the plant.

To address these issues, we have conducted a study, investigating the mechanism and dynamics of chemical uptake and bioaccumulation in *Myriophyllum spicatum*. This plant species was chosen because of its abundance in the Great Lakes, relative to other macrophyte species. Although our study investigates the chemical dynamics in *Myriophyllum spicatum*, we believe that our study also has significance for chemical uptake and bioconcentration in other macrophyte species because field studies revealed no interspecific differences within sites.

The primary objective of this study is to investigate the potential of macrophytes to be used as biomonitors. However, the study reported below is also useful for chemical fate modelling in the Great Lakes and for estimating chemical removal efficiencies in water treatment plants which use aquatic plants as "sorbing" agents.

Materials and Methods

Chemicals

Chlorobenzenes i.e. 1,3,5-tri-, 1,2,4,5-tetra-, penta-and hexachlorobenzene were obtained from Aldrich. PCBs, i.e. 2,2',5,5'-Tetra-, 2,2',4,4',6,6'-hexa-, 2,2',3,3',4,4',5,5'-octa-and decachlorobiphenyl, and octachlorostyrene were from Analabs. Analytical grade n-hexane, petroleum ether, 2,2,4-trimethylpentane and dichloromethane were obtained from Caledon, Ontario. Florisil 60/100 um Mesh and silicagel 100/200 um Mesh was from Supelco Canada Ltd. Anhydrous sodium sulphate, from J.T. Baker Chemical

Co., was heated to 650°C overnight and stored at 130°C before use.

Plants

Myriophyllum spicatum was collected from Goose Lake, Ontario. Experiment plants had an average wet weight of 9 g and a lipid content of 0.20 +/- 0.02 (std) %. During the experiment, the plants were freely floating in the water. They were kept in a submerged state at all times by attaching lead weights to the stems. No soil was present. Throughout the experiment, the plants were exposed to natural sunlight in Windsor, Ontario. During the experiments, the plants were actively photosynthesizing. The plants were growing as demonstrated by the formation of new leaves and adventitious roots. Plant growth was quantified by removing and successively weighing new leaves and roots. Over the entire uptake and depuration experiment plant growth was less than 5% of the plant's original weight. No plant decay was observed.

Experiments

Uptake

The uptake kinetics of the chemicals in the plants were determined in a continuous flow apparatus, previously described by Gobas et al. (1989). The continuous flow apparatus consisted of (i) a 150 L glass tank was filled with dechlorinated, aerated and carbon filtered Windsor tap water, an (ii) Asti teflon pump (Cole-Parmer Instrument Co.), and (iii) a generator column. The water was circulated from the tank, through the generator column and back to the tank at a flow rate of 60 L/hr. The generator column was prepared by coating 200 mg of tri-tetra and pentachlorobenzene, 100 mg of hexachlorobenzene, 50 mg of octachlorostyrene and

tetrachlorobiphenyl, 20 mg of hexachlorobiphenyl and 10 mg of octa- and decachlorobiphenyl onto 10 g of 60/80 um mesh hexane-washed Chromsorb. Before the plants were added, the water was circulated for 5 days. Then, 120 plants, with an average wet weight of 9 g, were placed in the tank. The temperature was 21 (+/- 1)°C. A water sample and three plants were collected after 0, 0.5, 1, 2, 3, 5, 7, 14, 21 and 25 days and analyzed individually.

Clearance

The kinetics of chemical elimination from the plants were determined by transferring the plants at the end of the 25 d uptake period to a 150 L glass tank, containing clean uncontaminated, carbon filtered water. Throughout the elimination period this water was continuously filtered by an activated carbon filter at a flow rate of 170 L/hr. Plant samples were taken after 0.25, 1, 2, 3, 5, 7, 14, 21, 28, 37, 61 and 133 days.

Analysis

Water Analysis

Water samples of 250 mL were taken from three different locations in the tank. They were immediately extracted, first, with 150 mL, and then twice with 75 mL petroleum ether. To remove dissolved water, the petroleum ether extract was passed through a 0.025 x 0.60 m column containing 20 mL sodium sulphate. Then, the extract was concentrated to 3 to 4 mL by evaporation (Buchner Rotavap). Clean-up was performed by passing the concentrated extract through a 0.025 x 0.60 m column, containing from top to bottom 10 mL sodium sulphate and 40 mL Florisil. The column was then washed with 200 mL petroleum ether. This extract was

concentrated to 1 to 10 mL by evaporation and then analyzed by gas chromatography. The recoveries of the entire analysis procedure, with exception of the actual water extraction, was determined with spiked petroleum ether samples. The recoveries ranged from 89 ± 3 % for trichlorobenzene to 97 ± 3% (n=3) for octachloro- and decachlorobiphenyl.

Plant analysis

(I) Extraction:

After sampling and weighing, each plant was cut individually in small pieces with a scissors. The pieces were transferred into a mortar together with 30 mL anhydrous sodium sulphate, and then ground to a powder. This powder was added to a 0.025 x 0.60 m column, containing, from bottom to top, glass wool, 10 mL anhydrous sodium sulphate and 70 mL (1:1) dichloromethane:petroleum ether. Then, another 10 ml anhydrous sulphate was added on top of the column. After 1 hr, the column was eluted with 250 mL dichloromethane:petroleum ether (1:1). Then, 2 mL 2,2,4-trimethylpentane was added to the extract, after which the extract was concentrated to approximately 1 to 2 mL.

(II) Clean up:

The concentrate was passed through a 0.01 x 0.55 m column, containing from bottom to top, 8 mL silica, 8 mL acidified silica (40% (w/w) sulphuric acid), and 3 mL anhydrous sodium sulphate. This column was eluted with 50 mL petroleum ether. The extract was then concentrated to 10 mL and analyzed by gas chromatography.

Lipid content

After extraction, but before clean-up, the plant extract was

evaporated to dryness and then further dried in an oven at 60°C for 1 hr. The lipids were then determined by weight.

Gas chromatography

Gas chromatographic analysis was performed on a Varian 3500, equipped with a 30m DB-5 capillary column (J&W Scientific), a ^{63}Ni electron capture detector, a Varian auto-injector and an integrator. Injector temperature was 250°C, detector temperature was 300°C, and column temperature was programmed from 50 to 300°C. Carrier gas was ultra high purity helium at 1.5 mL/min. Make-up gas was ultra high purity 5% methane-95% argon at 60 mL/min. The injection mode was splitless. Injection volume was 1 uL. Standards were prepared from the pure chemicals.

Results

At the start of the uptake period, the concentrations of all chemicals in the water, C_w (mol/m^3) were at their solubility levels. The only exception was decachlorobiphenyl with an initial concentration exceeding the aqueous solubility. This indicates that the measured decachlorobiphenyl concentration in the water may not truly represent actually dissolved chemical. After the plants were introduced to the water (i.e. start of the experiment), C_w dropped and then, after 3d, reached a constant or slowly declining concentration in the water during the remaining 22 d of the uptake period. This initial drop of the chemical concentration in the water was virtually absent for 1,3,5-trichlorobenzene, but it was larger for the higher K_{ow} chemicals. The largest drop from 0.58 to 0.032 ug/L was observed for decachlorobiphenyl. During the uptake period, the chemical concentrations in the plants, C_p (mol/m^3), increased with time and approached a constant concentration towards

the end of the uptake period. Figure 6.1 illustrates the results of a typical uptake experiment, i.e. the uptake of hexachlorobenzene in the plants. After the uptake period, when plants were transferred to clean, uncontaminated water, a loss of the chemical from the plants was observed with time. This is illustrated in Figure 6.2.

During the first 37 days, the concentrations of all chemicals in the plants dropped logarithmically with time, which is illustrated by the linear decrease of log C_p with time. During the remainder of the elimination period, the decrease of C_p tends to be somewhat slower than during the first 37 d, causing a deviation of the initial linear drop of log C_p with time. The largest drop of C_p with time was observed for 1,2,4,5-tetrachlorobenzene, the smallest drop for octachlorobiphenyl.

Developing A Descriptive Model

The observed uptake of the test chemicals from the water by the plants, followed by the decrease of the chemical concentration in the plant after the plants are transferred to clean water, suggests that the chemical uptake in the plants is a reversible process. The simplest description of this process is

$$C_p \text{ <-----> } C_w \qquad (1)$$

This model describes distribution of the chemical between two compartments i.e. the plants and the water, which are both considered to be homogeneous. Chemical transfer from the water to the plant is represented by a rate constant k_1, which has units of reciprocal time (d^{-1}). Chemical transfer from the plant to the water is characterized by a rate constant k_2 with units of d^{-1}. The

following differential equation describes the net flux of chemical F_p (mole chemical/time) between the water and the plant:

$$F_p = d(V_p.C_p)/dt = k_1.V_p.C_w - k_2.V_p.C_p \quad (2)$$

where $k_1.V_p.C_w$ or is the flux from the water into the plant, i.e. F_1 (mol/s) and $k_2.V_p.C_p$ is the flux F_2 (mol/s) from the plant to the water. To derive the relationship between the chemical concentration in the water and that in the plant, equation 2 should be integrated. This can be performed simply when the chemical concentration in the water, as well as the volume (or weight) of the plant and the rate constants for uptake and elimination are constant with time (i.e. no growth), resulting in

$$C_p = C_w.(k_1/k_2).\{1 - \exp(-k_2.t)\} \quad (3)$$

From equation 3 it transpires that, when exposed to a constant chemical concentration in the water, the chemical concentration in the plant increases logarithmically with time and then approaches a constant level i.e. $C_w.(k_1/k_2)$. The ratio of the uptake and elimination rate constants, i.e. (k_1/k_2), can thus be referred to as the plant-water bioconcentration factor K_{pw}.

Integration of equation 2 can also be achieved when the chemical concentration in the water is zero, such as during a typical depuration experiment when contaminated plants are exposed to clean water. Under those conditions integration of equation 1 gives

$$C_p = C_{p,t=0}.\exp(-k_2.t \quad (4)$$

or

$$\log C_p = \log C_{p,t=0} - k_2.t \quad (5)$$

where $C_{p,t=0}$ is the concentration in the plant at the beginning of the depuration period. Equation 4 demonstrates that the plant-water exchange model predicts an exponential decrease of the C_p with time during the depuration experiment.

The applicability of the reversible plant-water exchange model can be determined by fitting the model to the experimental data. This could have been simply achieved by fitting the observed increase of C_p with time to equation 2. However, this is only correct if during the experiment the chemical concentration in the water is constant and plant growth is insignificant. Plant growth, on a weight basis, was less than 5% over the 25 d uptake phase, and thus considered to be insignificant. But Figure 6.1 clearly demonstrates the variation of the chemical concentration in the water with time. The model was therefore fitted to the experimental data by a numerical integration procedure. This procedure derives the chemical concentration in the plant as the sum of increments in plant concentrations dC_p over time intervals dt, i.e. $C_p = \Sigma\ (dC_p)$. Each dC_p is calculated from equation 2, i.e.:

$$dC_p = (k_1.C_w - k_2.C_p).dt \qquad (6)$$

where (i) dt was chosen to be sufficiently small, (ii) C_w at every exposure time t, followed from water concentration measurements by fitting the observed water concentrations to a series of linear functions, which each connect the observed water concentration data at two consecutive exposure times, and (iii) k_1 and k_2 were selected to produce the best agreement between calculated and observed C_p. The best fit of the observed data was the one with the k_1 and k_2 value, for which the sum of the squared differences between calculated and observed C_p was the smallest. In this fashion, the uptake and elimination rate constants of all chemicals

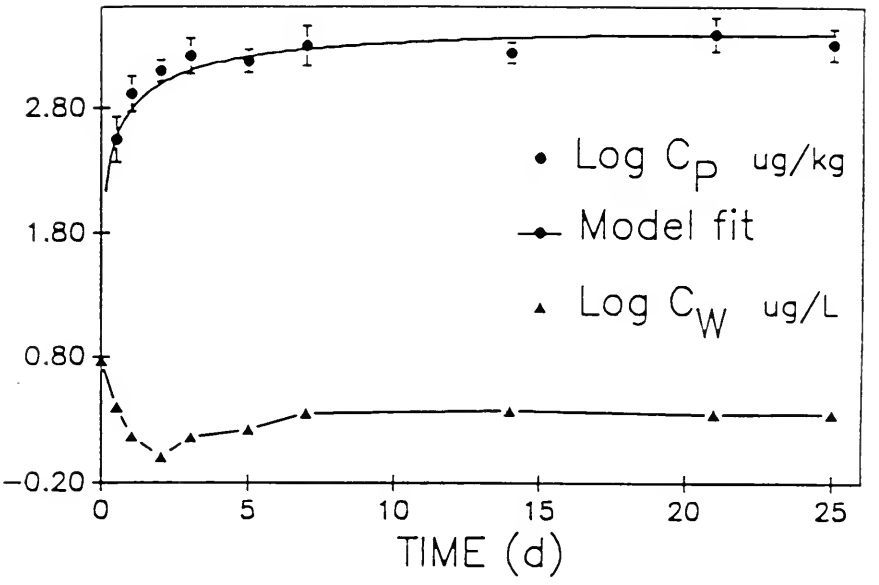


FIGURE 6.1

Logarithms of the concentrations of hexachlorobenzene in the water, C_w (ug/L), and in the plant C_w (ug/Kg) during the uptake experiment. The solid line illustrates the model fit.

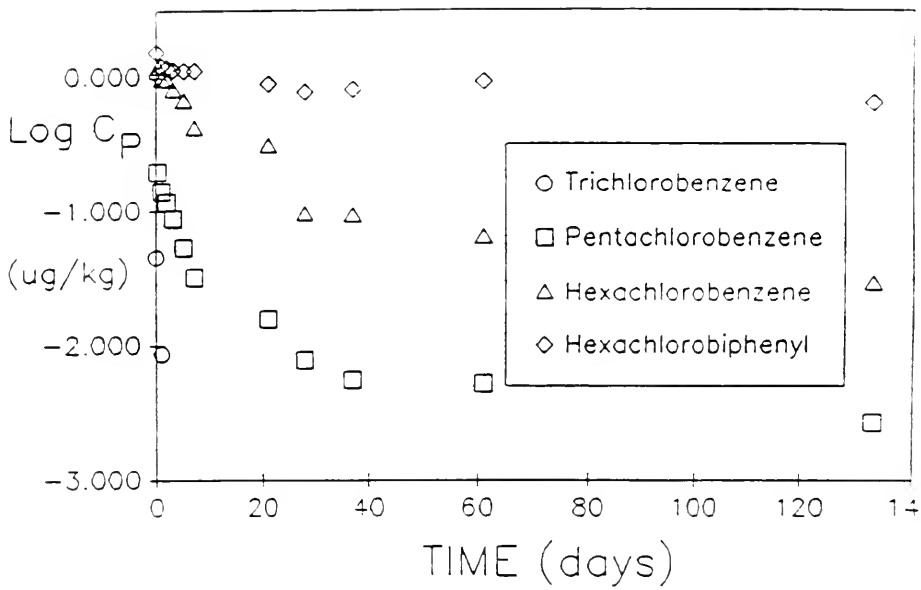


FIGURE 6.2

The logarithm of the chemical concentrations in the plant C_p (ug/Kg) during the elimination experiment.

were determined and listed in Table 6.1. The quality of the fit can be expressed by the normalized deviation, E, of the predicted (i.e. model) from the observed values

$$E = \Sigma_i \ (C_{Pii}^{\circ} - C_{Pii}^{M})^2/n \qquad (7)$$

where C_P° is the observed, and C_P^{M} is the predicted concentration in the plant and n is the number of observations. In this fashion it was estimated that for hexachlorobenzene, the observed and fitted plant concentrations vary, on average, only by 15%. This is well within the range of experimental error associated with plant and water analysis. In general, the deviation between observed and fitted plant concentrations ranged from 10 % to 45%. This demonstrates that the model agrees well with the experimental data.

Figure 6.2 demonstrates that the decrease of the concentration in the plant during the first 40 d of the elimination period is in agreement with the exponential drop of C_P (equation 4 and 5) predicted by the reversible plant-water exchange model. The rate constants for chemical elimination were thus determined from the slope of the log C_P-time plots. They are listed in Table 1. After the first 40 d of the elimination period, the decrease of the chemical concentration in the plant tends to be somewhat slower. This does not agree with the plant-water two compartment model. It could indicate that a small fraction of the chemical in the plant is less accessible than the majority of the chemical. However, it may also be due to experimental error associated with the long exposure times.

Table 6.1 demonstrates that the two independent measurements of k_2 (i.e. from uptake and elimination data) are in reasonable agreement. But, in general, the elimination rate constants derived

TABLE 6.1

The logarithm of the 1-octanol-water partition coefficient log K_{ow}, the uptake rate constant k_1 (d^{-1}), the elimination rate constant determined from the uptake data k_2^* (d^{-1}), the elimination rate constant determined from the elimination data k_2 (d^{-1}), the logarithm of the plant-water bioconcentration factor log K_{pw}, and the logarithm of the plant-water bioconcentration factor expressed on a lipid weight basis log K_{lw}.

Compound	logK_{ow}	k_1	k_2^*	k_2	logK_{pw}	logK_{lw}
trichlorobenzene	4.02	20	0.60	--	1.52	4.22
tetrachlorobenzene	4.51	93	0.54	0.16	2.24	4.94
pentachlorobenzene	5.03	275	0.20	0.092	3.14	5.84
hexachlorobenzene	5.47	150	0.14	0.069	3.03	5.73
tetrachlorobiphenyl	6.1	360	0.09	0.043	3.60	6.30
octachlorostyrene	6.29	403	0.065	0.03	3.79	6.49
hexachlorobiphenyl	7.0	580	0.02	0.012	4.46	7.16
octachlorobiphenyl	7.8	496	0.0008	0.0016	5.79	8.49
decachlorobiphenyl	8.26	486	0.0009	0.0029	5.73	8.43

from the elimination experiment (i.e. k_2) are approximately half of those derived from the uptake experiment (i.e. k_2^*). This discrepancy may be due to the fact that k_2 was derived from data over a 40 d period, while k_2^* was derived from data over a 25 d period. It is possible that when fitting data over the longer 40 d period, the influence of the relatively slow chemical transfer rate into less accessible plant compartments on the overall elimination rate is larger than when fitting over a shorter 25 d period. This could result in k_2 being somewhat lower than k_2^*.

Now that the uptake and elimination rate constants have been determined, it is possible to derive the bioconcentration factor of each chemical in the plant as k_1/k_2^*. The bioconcentration factors are listed in Table 6.1 and plotted versus the 1-octanol-water partition coefficient in Figure 6.3. Figure 6.3 demonstrates that the logarithms of the plant-water bioconcentration factor and the 1-octanol-water partition coefficients follow a linear relationship, i.e.

$$\log K_{PW} = 0.99\ (\pm\ 0.15).\log K_{OW} - 2.26\ (\pm\ 0.61) \qquad (8)$$

where the confidence intervals have a 95% probability. It suggests that chemical bioconcentration in the plant is essentially a chemical partitioning process which can be mimicked by 1-octanol-water partitioning. In fact, when the bioconcentration factor is expressed on a lipid weight basis as K_{LW}, i.e. the ratio of chemical concentration in plant lipids over that in the water, which is done in Table 6.1, it can be observed that bioconcentration factors and 1-octanol-water partition coefficients are approximately similar. This suggests that the chemical's affinity for the actual storage site in the plant is similar to that for 1-octanol. It also indicates that bioconcentration of the investigated chemicals in

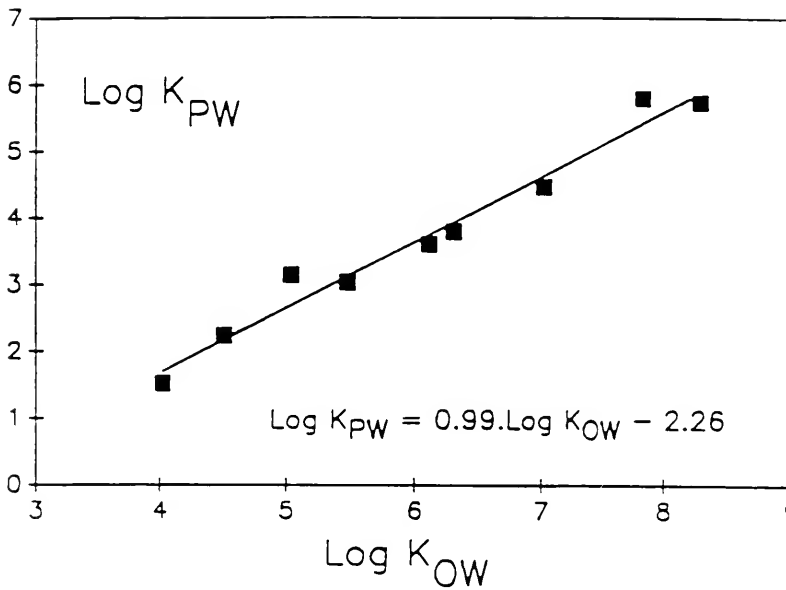


FIGURE 6.3

Relationship between the logarithm of the plant-water bioconcentration factor, log K_{PW}, and the logarithm of 1-octanol-water partition coefficient, log K_{OW}.

the plant is a thermodynamically controlled process, determined by the affinity of the chemical for the plant relative to that for water. It thus appears that uptake and elimination are passive processes, controlled by a thermodynamic gradient, and that there is no active transport.

Developing a Mechanistic Model

In Figure 6.4 the uptake rate constant is plotted versus the 1-octanol-water partition coefficient. It illustrates that for chemicals with log K_{ow} below 6.5, k_1 is increases with increasing K_{ow}, while for the chemicals with log K_{ow} exceeding 6.5, k_1 tends to approach a constant value of 500 d^{-1}. In Figure 6.5, k_2 is plotted versus K_{ow}. It shows that with increasing K_{ow}, k_2 drops, first slowly, but then more profoundly.

To explain this "biphasic" nature of the rate constant's relationship with K_{ow}, we propose a simple mechanism, which is based on the assumption that chemical bioconcentration in the plant involves chemical permeation through aqueous and organic parts of the plant. Examples of organic phases in the plants are the lipid bilayers of biological membranes or the plant's cuticle. Aqueous phases are present in several forms, for example, associated with membranes. In absence of active transfer mechanisms, transport of chemical from the ambient water to the storage site involves either simple molecular diffusion, movement through natural fluid flow in the plant, or both. When transport involves molecular diffusion, the chemical flux can be expressed by

$$F = k.A.\Delta C \qquad (9)$$

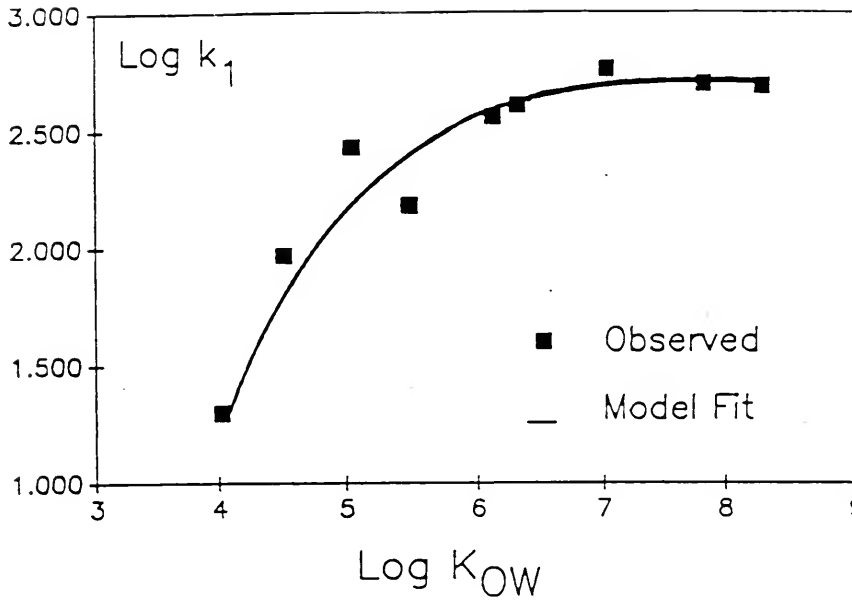


FIGURE 6.4

The logarithm of the uptake rate constant, log k_1 (d^{-1}), in the plant and the logarithm of the 1-octanol-water partition coefficient, log K_{ow}. The solid line represents the model fit.

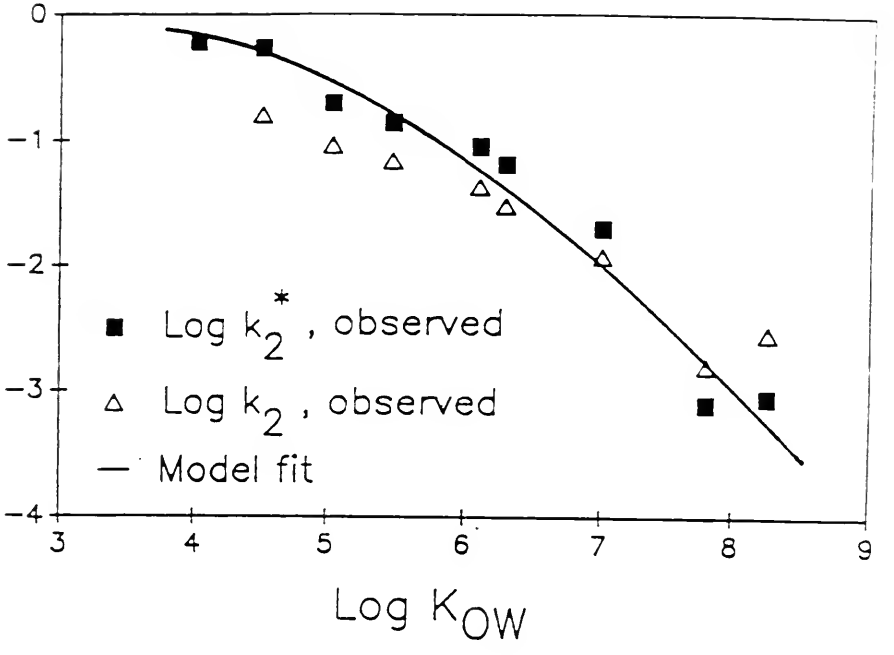


FIGURE 6.5

The logarithm of the elimination rate constant, log k_2 (d^{-1}), in the plant and the logarithm of the 1-octanol-water partition coefficient, log K_{ow}. The solid line illustrates the model fit.

where k is the mass transfer coefficient (m/s), A is the area of diffusion (m^2) and ΔC is the concentration gradient (mol/m^3).

If transport is by fluid flow, the flux is

$$F = Q.\Delta C \qquad (10)$$

where Q is the fluid flow (m^3/s). If both diffusion and fluid flow are involved in the transport process, it is possible to express the chemical flux as

$$F = D.\Delta C \qquad (11)$$

where D is the sum of all possible diffusion and fluid flow processes combined, i.e. D equals

$$D = \Sigma_i (k_i.A_i) + \Sigma_j Q_j \qquad (12)$$

D can be viewed as the chemical's conductivity, and its reciprocal, i.e. 1/D, as a resistance R (d). Since our experiment is not able to identify the mode of transport, i.e. diffusion, fluid flow, or both, involved in the bioconcentration process, we will refer to equations 11 and 12 as the best representation of the chemical flux. The chemical flux in aqueous phases of the plant, F_w (mol/s), is thus expressed as

$$F_w = D_w.\ \Delta C_w \qquad (13)$$

where D_w (m^3/s) is the chemical's conductivity in the water phases of the plant. The flux in the organic phases of the plant, F_L (mol/s), can be expressed as

aqueous and organic phase conductivities in the plant as:

$$1/k_1 = V_P/D_W + V_P/D_L.K_{LW} \quad (18)$$

In a similar fashion, k_2 can be expressed in terms of the aqueous and organic phase resistances. First, equation 17 is substituted in the equation for the overall chemical flux F_2 from the plant to the water, i.e.

$$F_2 = D_P.C_P/K_{PW} = D_P.C_P/(L_P.K_{LW}) \quad (19)$$

where K_{PW} is the bioconcentration factor in the plant, which, as was shown earlier, appears to be satisfactorily represented by the chemical's partition coefficient between plant lipids and water K_{LW} or by K_{OW}. If F_2 is then replaced by $k_2.V_P.C_P$, it follows that:

$$1/k_2 = L_P.V_P(K_{LW}/D_W + 1/D_L) \quad (20)$$

If K_{LW} in equations 18 and 20 are replaced by K_{OW}, it follows that

$$1/k_1 = V_P/D_W + V_P/D_L.K_{OW} \quad (21)$$

$$1/k_2 = L_P.V_P(K_{OW}/D_W + 1/D_L) \quad (22)$$

Equations 21 and 22 demonstrate that when chemical exchange between the plant and the water involves aqueous and organic phases in series (e.g. membrane permeation), the kinetics of uptake and elimination tend to be controlled by transport in the organic phases when the chemical's K_{OW} is low. With increasing K_{OW}, transport processes in the aqueous phases of the plant become more important and ultimately dominate the kinetics. This is reflected by (i) a k_1 which increases with increasing K_{OW} and then approaches a constant level (i.e. D_W/V_P) for high K_{OW}, and (ii) a k_2 which tends to be

$$F_L = D_L . \Delta C_L \quad (14)$$

where D_L (m^3/s) is the chemical's conductivity and C_L (mol/m^3) is the chemical's concentration in the organic phases of the plant.

In general, the solubility of organic substances in the organic phases of the plant are higher than that in the aqueous phases. The organic phase thus has a larger capacity to accommodate organic substances than the water. This difference is expressed by the chemical's organic phase/water partition coefficient K_{Lw}, i.e. C_L/C_w. It thus transpires that at all times the organic phase can transport a larger amount of chemical than the aqueous phase. This can be illustrated by expressing F_L in terms of C_w, i.e.

$$F_L = D_L . K_{Lw} . \Delta C_w \quad (15)$$

The overall chemical flux from the water into the plant, F_1 (mol/s), can be expressed as:

$$F_1 = D_p . \Delta C_w \quad (16)$$

where D_p (m^3/s) is the chemical's overall conductivity in the plant. If chemical transport in organic and aqueous phases occur in series, it follows that the total resistance for chemical uptake in the plant, R_p (i.e. $1/D_p$) is equal to the sum of the resistances in the aqueous, R_w (i.e. $1/D_w$) and in the organic phases, R_L (i.e. $1/D_L$):

$$R_p = 1/D_p = R_w + R_L/K_{Lw} = 1/D_w + 1/D_L . K_{Lw} \quad (17)$$

Substitution of equation 17 into equation 16, and replacing F_1 by $k_1 . V_p . C_w$ illustrates that k_1 can be expressed in terms of the

approximately constant (i.e. $D_L/V_P.L_P$) for low K_{ow} chemicals and then drops with increasing K_{ow}.

The applicability of this mechanistic model is demonstrated by its fit to our experimental data, resulting in :

$$1/k_1 = 0.0020 + 500/K_{ow} \quad (23)$$

$$1/k_2^* = 1.58 + 0.000015.K_{ow} \quad (24)$$

The quality of the fit is illustrated in Figures 6.4 and 6.5.

The good agreement between model and experimental data, provides the opportunity to estimate D_w/V_P and D_L/V_P. Comparison of equations 21 and 23, shows that D_w/V_P is 500 d and D_L/V_P is 0.0020 d. From equations 22 and 24, it follows that D_w/V_P is 133 d and D_L/V_P is 0.0013 d.

It is now interesting to discuss the significance of our findings in terms of a chemical transfer mechanism(s) in aquatic plants. Studies of the uptake of carbon dioxide and the release of oxygen indicate that simple passive diffusion is the predominant mode of transport. Our study also indicates that there is no active transport mechanism for the investigated organic substances. It is thus conceivable that chemical uptake and elimination in the plant is also the result of passive molecular diffusion. If diffusion is indeed the transport mechanism for chemical uptake and elimination, then D_w is $k_w.A$ and D_L is $k_L.A$, where k_w and k_L are the mass transfer coefficients in respectively the aqueous and organic phases of the plant and A is the diffusion area. It then follows that D_w is $k_w.A/V_P$ and D_L is $k_L.A/V_P$. Since our data indicate that D_w/V_P is between 133 and 500 d and that D_L/V_P is between 0.0013 and 0.0020 d, it is possible estimate k_w and k_L if the diffusion area per volume plant (i.e. A/V_P) can be determined. Measurements of the

actual diffusion area may be difficult. But it may be possible to estimate the diffusion area/volume ratio relationship. This may then render the aqueous and organic phase mass transfer coefficients, which may be too different among various macrophyte species. It may thus be possible to estimate the rate constants of chemical uptake and depuration in aquatic plants from the area/volume ratio of the plant. It is obvious that this is an hypothesis rather than a finding. Further research is required to demonstrate if there is sufficient basis that would allow rate constants of chemicals in aquatic macrophytes to be estimated from the area/volume ratio of the plant and the K_{ow} of the chemical.

Conclusions

(1) Our novel technique for chemical extraction from plant tissue makes it now possible to use aquatic macrophytes as biomonitors.

(2) Chemical concentrations in *Myriophyllum spicatum* simply reflect concentrations in the water through a bioconcentration factor, which can be estimated from K_{ow}.

(3) Steady-state or equilibrium is achieved rather quickly. As a result chemical concentrations in the macrophytes reflect ambient water concentrations over an historically short period of time. It thus provides exposure estimates of fairly recent contamination.

(4) Deployment periods, long enough to reach steady-state, can be determined from :

$$t_{eq} = 4.8 + 0.000045.K_{ow},\ t_{eq} \text{ in days}$$

For example, for hexachlorobenzene with K_{ow} of $10^{5.47}$, it takes approximately $4.8 + 0.000045.10^{5.47}$ i.e. 18 days for the plant to

reach a steady-state or equilibrium. If during these 18 days the concentration of hexachlorobenzene in the water does not vary considerably, the concentration of hexachlorobenzene in the water can be determined from the concentration in the plant as:

$$C_w = C_p/K_{pw}$$

where K_{pw} is the plant-water bioconcentration factor, which can be determined from equation 8, i.e.

$$\log K_{pw} = 0.99\ (+/-\ 0.15).\log K_{ow} - 2.26\ (+/-\ 0.61)$$

It can thus be concluded that *Myriophyllum spicatum* is an excellent *in-situ* biomonitor for organochlorines.

CHAPTER SEVEN

FINANCIAL REPORT

Disbursements associated with Grant 241PL (The Use of Aquatic Vegetation and Invertebrates to Monitor Chlorinated Hydrocarbons in the Lake Huron - Lake Erie Corridor) are summarized in Table 7.1

Most costs were associated with salaries of researchers, graduate students and technicians. Supplies purchased during the progress of the research were mainly associated with analytical work. There were no major equipment or computer purchases.

Table 7.2 summarizes staff and their responsibilities of completing the research presented in this final report.

TABLE 7.1

FINANCIAL SUMMARY
MAY 1986 - SEPT. 1989

Salaries & benefits	$186,174.00
Graduate Salaries	3,000.00
Supplies *	27,107.00
Postage	733.00
Printing	3,638.00
Small Equipment **	6,495.00
Telephone	1,865.00
Miscellaneous	2,845.00
Travel ***	14,383.00
	****$246,240.00

* Includes chemicals, carrier gas, glassware and repair of field equipment (boats).

** Includes purchase of capillary columns, sampling equipment and roto-evaporators.

*** Includes conference expenses (Technology Transfer Conference, IAGLR) and actual field expenses (gas, accommodations etc.)

**** An estimated cost of $4,000.00 is being held to publish the final report.

TABLE 7.2

PERSONNEL

Co-Principle Investigators *	-	G. D. Haffner
	-	P. D. Hebert
Research Scientists	-	F. A. Gobas
	-	D. Innes
	-	R. Lazar
Technicians	-	G. Allionte
	-	Y. Shah
	-	R. Kolasa
Graduate Students	-	B. Muncaster MSc
	-	E. Barycka MSc
	-	D. Bedard MSc
	-	C. Hebert MSc
Secretary	-	O. Fellet

* non-salaried

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APPENDIX A

ORGANOCHLORINE CONTAMINANTS IN DUCK POPULATIONS OF WALPOLE ISLAND

Abstract

The uptake of organochlorine compounds in liver and muscle tissues of resident and migratory ducks of Walpole Island was studied during 1986. Liver concentrations of octachlorostyrene (OCS) 115 ugKg-1, hexachlorobenzene (HBC) 30 ugKg-1 and pentachlorobenzene (QCB) 1.5 ugKg-1 were elevated in resident duck populations relative to migratory populations (56, 8.7 and .4 ug Kg-1 respectively). Comparison of residue concentrations in resident open water ducks (redheads) with resident marsh ducks (mallards) indicated that although there was no significant difference (p 71.0) between the two species, consistently higher levels in mallards might suggest these compounds are accumulating in the marshes.

The exposure of resident duck populations suggests that the transport and fate of organochlorinated compounds in the Huron-Erie Corridor are being regulated by physical and biological properties of the delta area. Low level discharges of persistent, bioaccumulating compounds into the upstream waters can result in an increasing concentration of these substances in the wetland area and in the associated food web.

Introduction

Walpole Island is a 24,000 hectare Indian Reservation located in the St. Clair River delta. The delta and the open waters of

Lake St. Clair are the second most important wetland system in the Great Lakes region (Herdendorf et al. 1986). Many species of birds, rare elsewhere in Ontario, are attracted to the productive wetlands (Goodwin 1982). These include many duck species which account for Lake St. Clair's reputation as an excellent hunting area. The native population of the island relies on this resource not only as a dietary supplement, but also as a valuable economic input into the local community through the hunting of ducks. Approximately 76% of the annual duck harvest in the area is comprised of mallards *Anas Platyrhynchos* (Jaworski and Raphael).

Since the discovery of pools of perchloroethylene in the St. Clair River in 1984, there has been concern as to whether other chlorinated compounds were moving downstream to the marshes and entering the food web. Of particular concern were compounds such as octachlorostyrene and hexachlorobenzene which are persistent and able to bioaccumulate. Octachlorostyrene (OCS), Hexachlorobenzene (HCB) and pentachlorobenzene (QCB) have previously been identified in Great Lakes fish (Kuehl et al. 1976, Kuehl et al. 1981). Suns et al. (1985) have also observed elevated levels of OCS and HCB in forage fish from the St. Clair River, and OCS has been previously identified in clams and sediments from the St. Clair River and Lake St. Clair (Kauss and Hamdy 1985, Pugsley et al. 1985, Oliver & Pugsley 1986). OCS has also been found in Lake St. Clair herons (Reichel et al. 1977). All three compounds, QCB, HCB, and OCS have the potential to bioaccumulate (Veith et al. 1979, Ernst et al. 1984) although QCB and HCB with lower Kow's might not bioaccumulate to the same extent as OCS. HCB, a known carcinogen, has been shown to cause adverse physiological effects in rats, humans, and Japanese quail (Ockner and Schmid 1961, Vos et al. 1971) It is also known to be toxic to herring gull embryos (Boersman et al. 1986). OCS is thought to be only moderately toxic (Gosselin et al.

1984, Tarkpea et al. 1985), but little is presently known of the environmental hazards of the compound. The New York Department of Environmental Conservation has set a consumption guideline for OCS at 20 ugKg-1.

A study was initiated in 1986 to determine if these substances were entering the food web of the Walpole marshes, and to determine which factors regulate the exposure of wildlife to organochlorinated contaminants discharged into the Huron-Erie corridor. Contaminant concentrations were determined for local and migratory populations of ducks to assess the severity of the contaminant problem of Walpole Island. The exposure of resident populations was studied by comparing residue levels of hens and their chicks. A final component of the study was to compare residue concentration in marsh and open-water ducks to determine if habitat selection and use could significantly affect exposure rates and subsequent body burdens.

Methods

Tissue Collections and Analysis

Forty-three ducks were shot by hunters from July to November 1986. These included 15 resident mallards (*Anas platyrhynchos*), 10 migratory mallards, 16 resident redheads (*Aythya americana*), and 2 migratory redheads. Migratory and non-migratory birds were distinguished from each other on the basis of leg coloration and plumage. One-migratory birds were collected during July and August groups of three: a mother and two chicks. Chicks were approximately 3 months old. Sites of collection (1-6) are indicated of Figure 1. Migratory birds were collected from October to November at sites A and B. Both the liver and the breast muscle

(without skin) were immediately removed upon collection. Tissues and frozen at 20oC until analyzed. All analyses were completed with 3 months of collection.

Tissue samples were thawed and 5.0 g of liver tissue of 10.0 g of muscle tissue was homogenized in 120 ml of acetonitrile (distilled-in-glass-caledon) and 40 ml of water using a Polytron for 1 - 1.5 minutes. The homogenate was filtered with suction through a sintered glass funnel. The process was repeated three times, and the combined filtrates were transferred into a 2000 ml separatory funnel. Concentrated sulphuric acid (1 mL) was added and the resultant mixture was then back-extracted using 300 mL of petroleum ether (Caledon) in three portions of 150 mL, 75 mL, and 75 mL. The combined petroleum ether extracts were washed with 200 mL of distilled water and dried by passage through columns containing 15 g of activated anhydrous sodium sulphate. The dried extracts were concentrated to 5 mL using a Kuderna-Danish evaporator. The concentrate was added to 20 mm x 40 cm glass columns containing 30 g of activated Florisil (Sulpelco) topped with a 1 - 2 cm layer of anhydrous sodium sulphate. The column was eluted with 200 mL of petroleum ether and the eluate was concentrated in a kuderna-Danish evaporator to 4 - 5 mL. The concentrate was diluted to 10 mL with pesticide grade hexane and 1.0 uL was injected. This method resulted in recovery efficiencies of 60%, 72% and 90% for QCB, HCB and OCS respectively. Literature values for the log Kow for each compound are as follows: QCB = 4.9, HCB = 5.5, and OCS = 6.2 (Oliver 1987).

A Hewlett-Packard Model 5790A capillary column GC-ECD fitted with a 30 m x 0.25 mm DB-5 capillary (J and W Scientific) was used for the analyses. Each set of five samples analyzed was also accompanied by a solvent blank which had undergone the entire

isolation procedure along with associated standards (QCB, HCB-ChemService, OCS-, Health and Welfare Canada). The limit of quantification for QCB, HCB, and OCS was 0.2 ug kg-1. When analyzed samples resulted in a non-detectable value (ND) then a zero value was used in calculating the reported statistics. Values were not corrected for recovery efficiencies.

Lipid Extraction

Lipid extracts from muscle and liver samples were obtained using the initial procedure described above. However, during the petroleum ether extraction, sulphuric acid was not added. Combined petroleum ether extracts were evaporated in a vacuum chamber and percent lipid was the calculated.

Results

The three contaminants (QCB, HCB and OCS) were frequently detected in the samples analyzed. Concentrations of OCS were the highest of the contaminants examined in duck tissue as would be anticipated considering its higher Kow and potential to bioaccumulate. A one-way ANOVA showed that liver tissue had significantly higher residue levels in the liver, liver tissue was used to compare contaminant burdens between resident and migratory duck populations. Lipid levels in liver tissue were low (0.4-1.6%).

A one-way ANOVA indicated that all three contaminants were more concentrated ($P<0.05$) in tissues of resident birds than in those of migrants (Figure 2 & 3). Standard errors of the estimated mean values, as illustrated in Figures 2 & 3, were much reduced in migratory populations particularly for QCB and HCB.

A comparison of residue concentrations in livers of hens and chicks was made using a matched pair 't' test. There was no significant difference ($P>0.10$) between hens and chicks, suggesting that exposures over a three month period were sufficient to account for the measured body burdens. Since contaminant levels in the eggs were not examined, it is impossible to state how much of the chicks body burden resulted from maternal inheritance. A correlation of contaminant concentrations between hens and chicks, as shown in figure 4, indicates considerable variation of contaminant burden within families of the non-migratory populations. Although contaminants might be maternally inherited, this process would not explain the considerable variability observed between siblings, particularly considering the low variability in the OCS levels observed between siblings.

There was no significant difference in the contaminant burdens of redhead and mallard ducks ($P>0.1$). Nevertheless, Figure 5 demonstrates a consistent pattern; for each of the contaminants studied the mean body burden in mallard ducks was greater than that for redhead ducks. The similarity in the levels between species of QCB, HCB and OCS might reflect the overall importance of the octanol - water partition coefficient in regulating body burdens. The low Kow compound, QCB, showed little accumulation, whereas OCS illustrated considerable uptake.

Discussion

The levels of all contaminants observed in this study indicated that resident duck populations were being exposed to a higher degree of contamination than migratory populations. This suggest that the St. Clair River - Lake St. Clair ecosystems continues to be exposed to these contaminants particularly in the

area of the Walpole Marsh. A local source of OCS was from an industrial chlorine manufacturer in Sarnia. The transport of OCS through the ST. Clair River and into Lake St. Clair was previously documented by Pugsley et al. (1985). Sources of HCB and QCB contamination are also known to be present in this highly industrialized area. Recent loading reductions of HCB have been reported to be a as high as 80% (MOE, unpublished).

There was no significant difference in contaminant levels between resident redhead and mallard ducks. This may be accounted for in that ducks from these populations were collected during the nesting period (July - August). It is probable that there was considerable habitat overlap between the two species at this time, in that the redhead exhibits an unusual feeding behaviour for a diving duck. Redhead ducks forage in shallower waters and feed more extensively on aquatic plants than other diving duck species. The diet of a redhead duck has been shown to consist of approximately 90 percent vegetation and 10 percent animal life (Bellrose, 1976). In this respect, resource utilization by the redhead is similar to the mallard, a dabbling duck. The mallard's diet consists solely of vegetation (Bellrose 1976). It may be these similarities in feeding behaviour that result in the similar contaminant levels in the two species particularly for the higher Kow compounds.

Mallard ducks, however, tended to have consistently greater contaminant levels that redhead ducks possibly due to the fact that the marsh is acting as a chemical sink. Considering the hydrophobic nature and stability of these compounds they might collect and persist in organic sediments (Oliver & Pugsley, 1986). Compounds with relatively high Kows such as OCS have been shown to have high absorption potentials (Ernst et al. 1984). Polluted

sediments may then act as reservoirs of "in-place" contaminants which continue to enter the food chain long after the primary sources of contamination have been removed (Smith et al. 1985). The mallard's greater exposure might be a function of its "dabbling" feeding behaviour which results in a closer association with contaminated sediments. This might explain the consistently higher contaminant levels in the mallards.

Conclusion

As there are no Canadian guideline levels for consumption of compounds such as OCS, HCB and QCB, it is difficult to predict what effect long-term exposures might have on humans using the ducks as a food resource. Native populations of Walpole Island are of particular concern due to their dependence of "wild" foods for a large proportion of their diet. There is considerable evidence, however, that contamination of resident duck populations is a current problem, and probably associated with 'in place' contaminants and foodweb transfers. Further studies would be required to resolve the pathways of exposure for the different compounds of concern. Considering levels of OCS were well above the NYDEC consumption guideline, there is a need to assess the risk of exposure of the native population to OCS. This is a particular concern given the dependence of this population on local fowl and fish populations.

Acknowledgements

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List of Tables

Mean and Std. Deviation	Mean	Mean (ug/kg)		
	Wet weight of sample (g)	OCB	HCB	OCS
Non-migratory Mallards(N=15)				
Liver	6.72	1.53	29.59	115.33
	±2.49	±1.51	±30.79	±93.22
Muscle	10.02	0.55	07.14	11.71
	±0.41	±0.38	±5.29	±36.20
Migratory Mallards (N=10)				
Liver	4.80	0.41	08.70	56.08
	±0.74	±0.46	±16.47	±93.66
Muscle	09.63	0.47	4.82	1.67
	±0.94	±0.23	±10.75	±2.13
Non-migratory Redheads (N=16)				
Liver	5.76	1.18	20.01	85.60
	±2.28	±1.47	±28.57	±102.64
Muscle	09.82	0.77	10.76	4.78
	±1.48	±0.78	±15.91	±4.43
Migratory Redheads (N=2)				
Liver	4.90	0.49	2.04	18.93
	±0.24	±0.13	±1.44	±15.30
Muscle	10.80	0.82	1.66	3.49
	±0.78	±0.61	±1.19	±4.02

TABLE 1

Walpole Island ducks 1986 - means and standard deviation

List of Figures

SITES OF COLLECTION

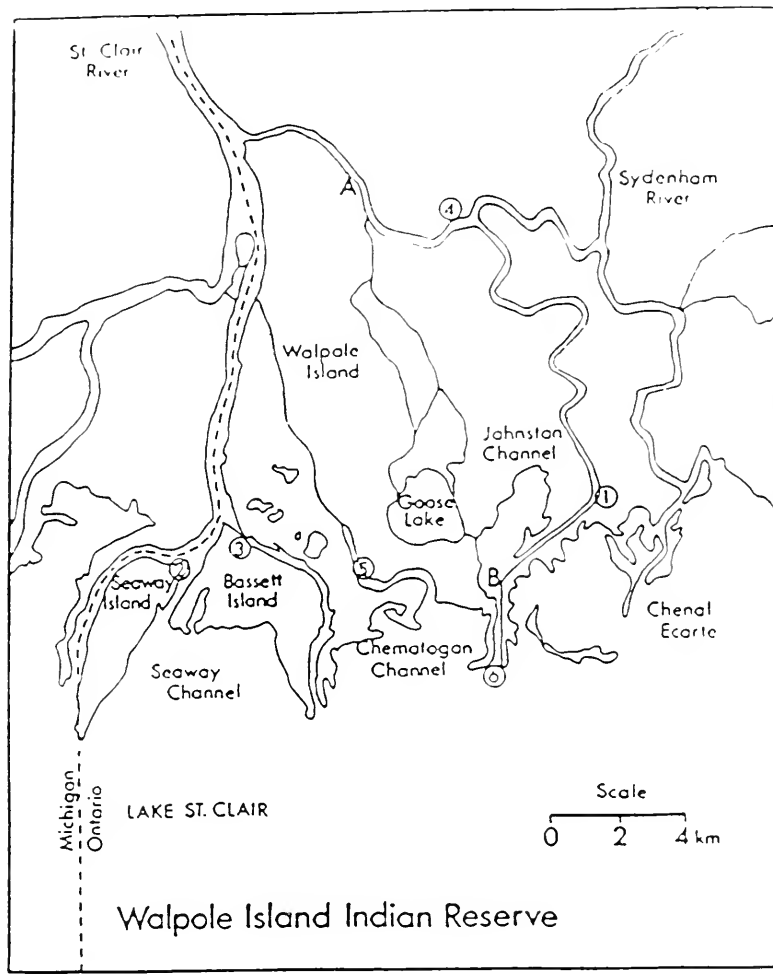

FIGURE 1

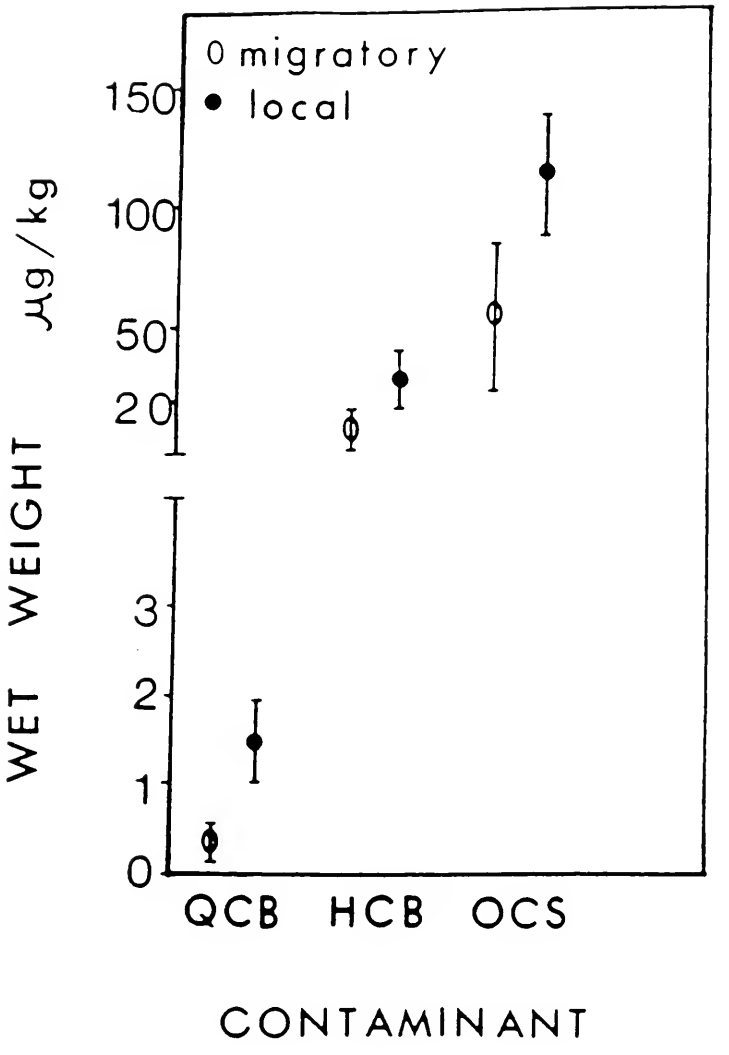
0 migratory
● local
150
100
50
20
3
2
1
0
WET WEIGHT µg/kg
QCB HCB OCS
CONTAMINANT

FIGURE 2

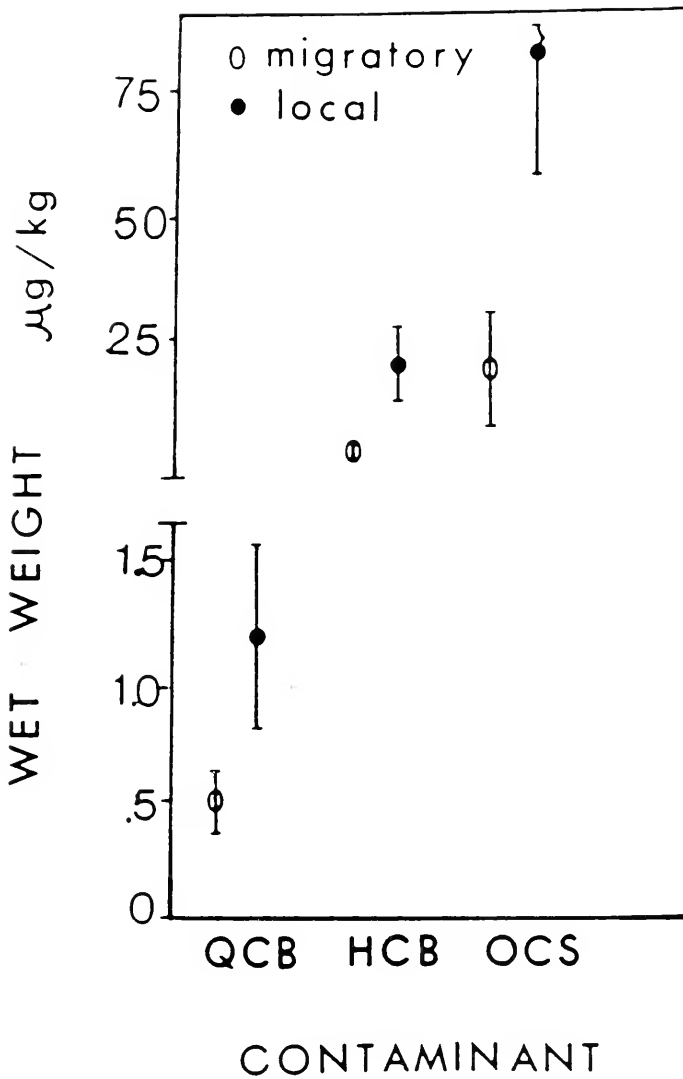
o migratory
• local
75
50
25
1.5
1.0
.5
0
WET WEIGHT µg/kg
QCB
HCB
OCS
CONTAMINANT

FIGURE 3

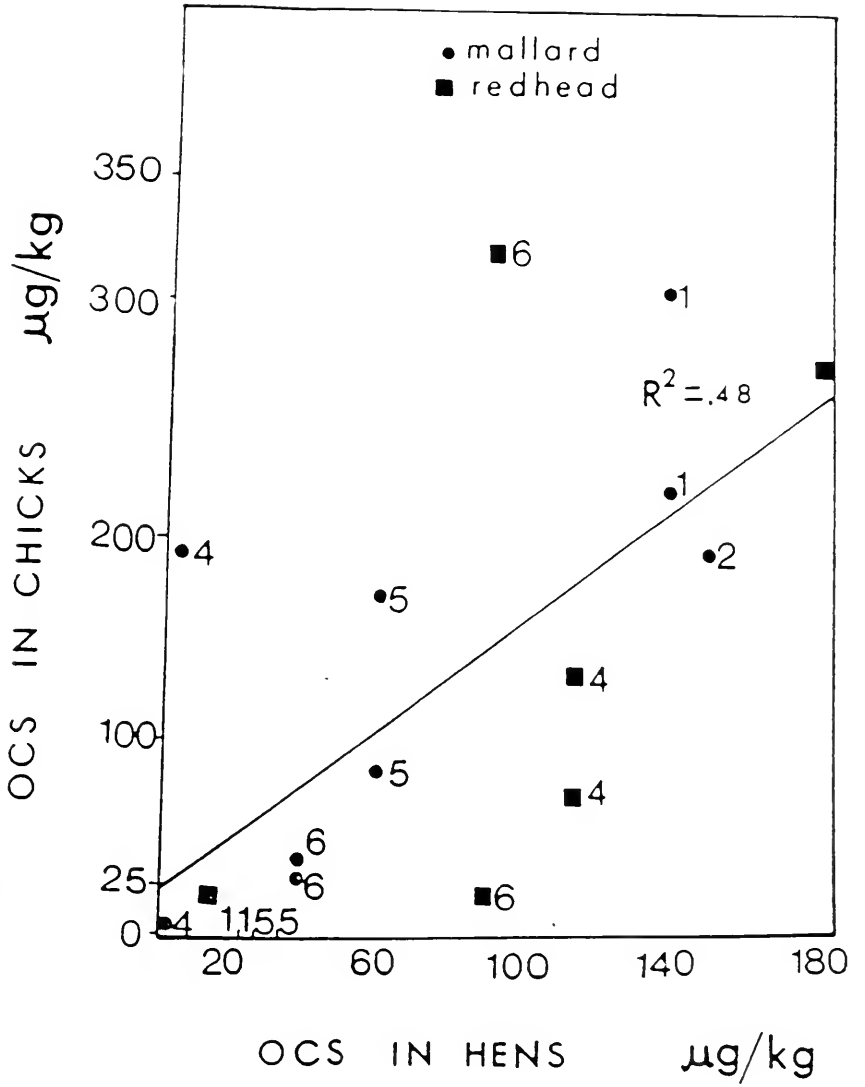
• mallard
■ redhead
350
300
200
100
25
0
µg/kg
OCS IN CHICKS
R^2 =.48
20
60
100
140
180
OCS IN HENS µg/kg

FIGURE 4

APPENDIX B

HABITAT PARTITIONING AND CONTAMINANT EXPOSURE IN FORAGE FISH

Abstract

Four species of forage fish: *Labidesthes sicculus*, a surface feeder; *Notropis atherinoides*, a facultative surface feeder; *Notropis hudsonius*, a facultative benthivore; and *Pimephales notatus*, a benthivore were collected during 1987-88 from sites in the St. Clair and Detroit Rivers. Whole body levels of pentachlorobenzene, hexachlorobenzene, octachlorostyrene and PCB congeners 31, 52, 87, 101, 118, 138, 153, and 180 were determined. Significant interspecific differences in contaminant concentrations were observed for compounds with a log Kow greater than 6.0. Highest mean contaminant levels were seen in *P. notatus* and lowest levels were observed in L. sicculus. It was noted that food and habitat utilization were important factors regulating body burdens in these fish species.

Key Words: forage fish, organochlorines, biomagnification.

Introduction

Debate exists concerning the role that biomagnification plays in regulating contaminant distributions in aquatic ecosystems. The thermodynamic approach, based on fugacity models, predicts that organisms in equilibrium with their environment should show similar levels of contaminants in their lipid fractions, regardless of their trophic status or habitat utilization (Connolly and Pederson 1988). The fugacity approach divides the environment into phases

such as biota, sediment, suspended solids, water, and air. If transfer of a chemical is to occur passively among these compartments, then a gradient in chemical potential, or fugacity, must exist. At equilibrium, the fugacity of all phases is assumed to be equal with no net transfer of contaminants. In such a balanced aquatic ecosystem one would not predict any differences in lipid-normalized contaminant levels among aquatic organisms.

When differences in contaminant levels are observed between trophic levels, these differences are relatively small and only occur for a few very hydrophobic compounds (Thomann and Connolly 1984). Such differences have also been attributed to non-equilibrium conditions which assume that organisms are moving towards equifugacity, possibly at different rates, but have not yet reached that state. Non-equilibrium dynamics, for example, would also result from the slow uptake rates of highly hydrophobic compounds compared with actual organism growth rates. Therefore, large species at higher trophic levels would have the greatest chemical body burdens not only because feeding provides additional exposure to the chemical but also because exposure duration would be greatest and chemical elimination would be slowest for these species.

Other work has suggested that an alternate model based on food web dynamics can better explain the distribution of contaminants in aquatic ecosystems. Borgmann and Whittle (1983) illustrated that larger organisms had higher contaminant levels than smaller organisms, but as their data were not lipid normalized it is not possible to demonstrate biomagnification. Their results did suggest, however, that biomagnification could occur. Thomann and Connolly (1984) developed an age-dependent food chain model for polychlorinated biphenyls in the Lake Michigan food chain and

concluded that the major route of PCB uptake was from food. This mechanism was further substantiated by Oliver and Niimi (1988) who demonstrated the importance of 'trophodynamics' in contaminant partitioning in the Lake Ontario ecosystem. Similar observations have been made by Connolly and Pederson (1988) and Gobas et al. (1988), resulting in considerable evidence that food resources play an important role in determining the distribution and levels of selected organic contaminants in aquatic food webs.

If food is an important exposure route, then differences in resource utilization among species can play an important role in regulating their contaminant body burdens (Flint et al. 1988). To further resolve the factors regulating chemical levels in organisms, we examined contaminant levels in forage fish. Within this component of the foodweb, benthic, pelagic, and allochthonous foodwebs are differentially utilized by philopatric species of similar size and age. Should chemical properties, related to the bioconcentration potential of contaminants, be singularly important (Neely et al. 1974, Veith et al. 1979) one would expect negligible interspecific differences amongst these organisms. Similarly, other chemical factors such as aqueous solubility, which regulates the bioavailability of a compound, should also result in equivalent body burdens. Should, however, differences in lipid normalized contaminant levels be observed, other regulating factors would need to be examined. Such factors might include metabolic differences and/or differences in exposure dynamics.

Few studies have examined the relative importance of ecological factors in regulating contaminant levels and distribution in aquatic food webs. Many species, for which ecological data are lacking, have been considered by monitoring programs to be ecological equivalents. As a result, different biomonitors are

often expected to integrate changes in contaminant levels in a similar manner. This assumption might not be justified for organisms that differ either ecologically or metabolically.

Forage fish populations provide an opportunity to test this assumption. Within this group, minor morphological differences among species can correspond to major differences in habitat utilization (Keast and Webb 1966). Forage fish are useful biomonitors in that they have limited home ranges and are accurate indicators of the local bioavailability of contaminants (Hebert and Haffner 1989a). For example, the spottail shiner (*Notropis hudsonius*), has been used to monitor the spatial distribution of contaminants in the Great Lakes as well as to evaluate long-term trends in contaminant levels (Suns et al. 1985, Environment Ontario 1985). However, little is known regarding the factors that regulate contaminant accumulation in this species or if this species represents contaminant levels in forage fish in general. In this study, the relative importance of physiological, chemical, and ecological factors in determining contaminant burdens in forage fish species is examined.

Materials and Methods

Four species of forage fish were examined in this study (Figure 1): *Labidesthes sicculus* (Cope) (brook silverside), *Notropis atherinoides* Rafinesque (emerald shiner), *Notropis hudsonius* (Clinton) (spottail shiner), and *Pimephales notatus* Rafinesque (bluntnose minnow). *N. atherinoides* is not shown in Figure 1 but is similiar morphologically to *N. hudsonius*. However, there are major anatomical differences between surface, water-column, and bottom feeders. These physical differences determine their partitioning of food resources (Keast and Webb 1966). L.

sicculus is primarily a surface feeding species (Scott and Crossman 1973) with a beak-like snout and dorso-terminal mouth designed to seize prey at the surface. Stomach contents reveals that its diet consists primarily of small flying insects and some cladocerans (Scott and Crossman 1973). *N. atherinoides* has a terminal mouth and is a facultative surface feeder whose diet consists primarily of zooplankton (Scott and Crossman 1973). *N. hudsonius* has a terminal mouth, but analysis of gut contents, as summarized in Scott and Crossman (1973), indicates that this species consumes a wide variety of organisms including cladocera, aquatic insect larvae, and algae. It could therefore be classified as a facultative benthivore. *P. notatus* possesses a ventro-terminal mouth designed for benthic feeding (Scott and Crossman 1973), and subsists almost entirely on chironomid larvae and organic detritus from the bottom. These species represented an integration of allochthonous, pelagic, and benthic foodwebs integral to the purposes of this study. Gut contents were examined in *P. notatus* and *L. sicculus* to verify this resource partitioning. The partitioning of food resources between *N. hudsonius* and *N. atherinoides* has been previously documented. In a study by Muth and Busch (1984), there was little dietary overlap between the two species with *N. atherinoides* mainly feeding on cladocera. As these two species are generalists in the sense they both feed in both the benthic and pelagic food webs, it would be expected that specialists such as *P. notatus* and *L. sicculus* would have pronounced differences based on their morphological adaptations to feed only in the benthic and pelagic food webs respectively.

Fish were collected from two sampling locations. Site 1 was located in the St. Clair River approximately 1.5 km south of Sarnia's Chemical Valley. It was chosen due to its proximity to important sources of chemical contamination on the Canadian side of

the river. Water was shallow (1-1.5 meters) and well mixed at this site. The sediment consisted of a mixture of silt and coarse sand. A second site, located in the Trenton Channel of the Detroit River near the southern tip of Grosse Isle was chosen to compare chemical distribution with the St. Clair River system.

Site 1 on the St. Clair River was sampled during August and September of 1987. Young-of-the-year of the 4 fish species were caught in nearshore waters using a 10 m, 0.6 cm mesh bagseine. Immature fish were collected to minimize intersexual differences. They were measured (total length), grouped according to length and immediately wrapped in hexane-rinsed aluminum foil. Samples were kept frozen at -20 degrees Celsius until they were analyzed. This site was sampled again in September 1988 and at this time both fish and sediment samples were collected. The top 3 cm of sediment was collected and placed in hexane-rinsed amber glass jars. These jars were stored at -20 degrees Celsius until the sediment was analyzed.

During October of 1988 (Figure 2) two species of fish, *L. sicculus* and *P. notatus*, were collected from Site 2 on the Detroit River. Sediment samples were also collected as previously described.

A variety of compounds were examined, in fish and sediment, which varied over two orders of magnitude in their 1-octanol water partition coefficients (Kow). Pentachlorobenzene (log Kow = 5.2), hexachlorobenzene (5.5), octachlorostyrene (6.3) and PCB congeners 101 (6.38) and 180 (7.36) were examined at both sites. In addition, at the Detroit River site, PCB congeners 31 (log Kow = 5.7), 52 (5.84), 87 (6.29), 118 (6.74), 138 (6.83), and 153 (6.92) were examined. Fish from the St. Clair River site had low levels of PCBs therefore the wide range of congeners was not examined in 1987. In

1988, however, all of the compounds were examined so that complete comparisons could be made between the St. Clair and Detroit River sites.

Whole fish were analyzed according to extraction and clean up protocols developed by the Canadian Wildlife Service (1982). All samples consisted of approximately five individuals and the composite wet weight was not less than three grams. The only deviation from CWS methodology was that during the Florisil column clean-up, petroleum ether (Caledon, distilled-in-glass) was used as the eluant instead of hexane.

Sediment samples were analyzed using soxhlet procedures. For all samples, only the first fraction from the Florisil column was collected. This fraction contained chlorobenzenes, octachlorostyrene, and various PCB congeners. Recovery efficiencies for this method were determined to be 87% for QCB, 89% for HCB, and 91% for OCS. Recovery efficiencies for the PCB congeners were measured to be 90%. Reported values were not corrected for recovery efficiencies. To determine if the existing gut content could affect body burden, gutted fish were analyzed and compared with body burdens of whole fish samples. Lipid determinations were made on each sample. The organic carbon content of each of the sediment samples was determined using loss on ignition.

The samples were injected into a Hewlett-Packard Model 5790A GC-ECD fitted with a 30m x 0.25mm DB-5 capillary column (J+W Scientific). Standards (QCB,HCB-Chemservice, OCS-Health and Welfare Canada, PCB congeners-Environment Canada) and a solvent blank, which had undergone the entire isolation procedure, accompanied each set of six samples. For QCB,HCB,and OCS the limit of quantification was 0.1 ug kg^{-1}. For the PCB congeners the limit of

quantification was 0.2 ug kg^{-1}.

Results

Figure 3 illustrates the lipid-normalized body burdens of pentachlorobenzene (QCB), hexachlorobenzene (HCB), octachlorostyrene (OCS), and PCB congeners 101 and 180 in the four species from Site 1. It is apparent that considerable interspecific compartmentalization of the contaminants occurs. Figure 4 confirms this pattern of chemical compartmentalization for *L. sicculus* and *P. notatus* from the Detroit River site. In both the St. Clair and Detroit Rivers *P. notatus* had the highest mean contaminant levels and *L. sicculus* the lowest. In the St. Clair River mean contaminant levels in *P. notatus* were followed by *N. hudsonius* and then by N. atherinoides. It is also evident (Figures 3 and 4) that as the log Kow of the compounds increases these interspecific differences become more significant. No significant differences in length or lipid content were observed among the species (ANOVA $p>0.1$). Therefore, contaminant distribution in these species is not a function of allometric differences amongst them. Contaminant burdens in samples containing gutted P. notatus were not significantly different from comparative samples consisting of whole fish (ANOVA $p>0.1$). Analysis of gut contents revealed that the diet of *L. sicculus* consisted primarily of prey associated with surface waters. *P. notatus*, however, fed primarily on benthic organisms (Figure 5).

Bioaccumulation factors (BAFs) were estimated for each of the species at both sites during 1988 in order to determine the relationship between contaminant levels in the fish and the sediment and the 1-octanol water partition coefficient. These BAFs were calculated by dividing the lipid-normalized contaminant levels

in the fish by the organic carbon normalized contaminant levels in the sediment. Figure 6 shows a plot of log BAF versus log Kow for three of the four species at the St. Clair River site. These fish were collected in September of 1988 along with sediment from that site. *N. atherinoides* was not present in the collection at that time and therefore, is not shown in the figure. There is a linear relationship between log BAF and log Kow for the three species (*L. sicculus*, r^2=0.81; *N. hudsonius*, r^2=0.88; *P. notatus*, r^2=0.93). An analysis of covariance indicated that the slopes of these lines are not significantly different ($p>0.1$), but the intercepts do differ ($p<0.05$). *P. notatus* and *N. hudsonius* had significantly greater BAFs than *L. sicculus*. Figure 7 shows a plot of log BAF versus log Kow for *L. sicculus* and *P. notatus* collected from the Detroit River site. *P. notatus* again had higher BAFs than those determined for *L. sicculus* but only for the higher Kow compounds. A linear relationship exists between log BAF and log Kow for both species (*L. sicculus*, r^2=0.45; *P. notatus*, r^2=0.64). An analysis of covariance indicates that there is no significant difference in the slopes of the lines for the two species or in their intercepts ($p>0.1$).

Discussion

Past efforts to define the major routes of chemical movement in aquatic food webs usually involved integrating various independent studies. This study is among the first to present evidence of interspecific differences in contaminant levels in species of fish of the same age, size and lipid content. As suggested in earlier studies, food can play an important role in determining contaminant levels and distribution (Thomann and Connolly 1984; Oliver and Niimi 1988). When sediment or sediment-associated organisms are an important component of the diet,

exposure via food can be of particular importance (Schindler 1987). The role that sediments have in contaminant transfer has often been downplayed due to the assumption that chemicals associated with sediments are highly bound, and therefore not biologically available (Neff 1984). In most ecosystems, however, many physical and biological processes may re-introduce these compounds into aquatic food chains (Renyoldson 1987, Riedel 1987). Previous studies have shown the potential for contaminant uptake from sediments by invertebrates (Oliver 1984, Larsson 1986, Oliver 1987) and fish. Larsson (1986) concluded that sediment-mediated uptake by fishes could occur by two processes: 1) bioturbation, desorption, and gas convection allowing contaminants to move across the sediment-water interface and be bioconcentrated and 2) food chain transfer which initially involves bioaccumulation by benthic invertebrates followed by transfer to predatory fishes. Other studies have indicated that contaminant transfer to fish need not be limited to a food-chain mechanism but that accumulation can occur directly from the sediment by detritivores (Seelye et al. 1982, Willford et al. 1987).

The interspecific differences in contaminant burdens observed in this study confirm that food and habitat selection, are major factors regulating contaminant levels in forage fish. In the St. Clair River, interspecific differences were associated with the higher Kow compounds only. There were no significant differences, among levels of low Kow compounds such as pentachlorobenzene, which would be relatively water soluble compared with the other compounds studied. This was further substantiated by the results obtained at the Detroit River site. A wider range of compounds were examined and no interspecific differences were seen between the surface and benthic feeders for any chemical with a log Kow less than 6.0. This pattern confirms that for compounds with a log Kow less than 6.0

exposure through water is the primary route of transfer. Exposure to chemicals through water would be more homogeneous than through food because low Kow compounds would be uniformly distributed in the aqueous phase whereas individual prey items may vary in their chemical concentrations. High Kow compounds with low aqueous solubility, such as OCS and PCB 180, tend to be associated more with the particulate (food) phase than the water phase. When feeding, *P. notatus* is in continual contact with the sediment substrate and may ingest a substantial amount of organic detritus with its food. As a result of feeding on benthic organic detritus and zoobenthos, *P. notatus* had consistently higher levels of the higher Kow contaminants. *N. hudsonius*, a facultative benthivore, also had significantly higher contaminant burdens than *L. sicculus* and *N. atherinoides* but lower levels than *P. notatus*. This might be due to its selection of a wider variety of food items and less dependence on organic detritus, than *P. notatus*. The levels of contaminants in *L. sicculus* remained much lower than in the two benthic species because a large proportion of its diet consists of terrestrial insects with much lower HCB and OCS levels (Kovats et al. 1989). There is also little or no direct contact between *L. sicculus* and the organic carbon pool of the sediments.

Bioaccumulation factors illustrate that the species associated with the benthic community had higher BAFs than the surface feeding species. There was no significant difference in the slopes of the lines for any of the species which support the assumption that the uptake and depuration mechanisms regulating bioaccumulation are similar. Thus it is evident that interspecific differences in metabolism of these compounds are negligible. This is corroborated by other work which shows that chemical elimination rate constants for QCB, HCB, and OCS are similiar for *L. sicculus* and *P. notatus* (Hebert and Haffner 1989b). The different BAFs observed among

species of forage fish reflect that exposure dynamics are species dependent.

At the St. Clair River site, significant differences were observed between the intercepts of the lines for the benthivores versus the surface feeder. The Detroit River site, however, showed no significant difference between the intercepts of *P. notatus* and *L. sicculus*. The latter is what would be predicted for the lower Kow compounds as exposure to these less hydrophobic compounds should be more homogeneous with water being the primary source of contamination (Haffner et al, 1990). The interspecific differences for low Kow compounds observed at Site 1 may be a result of the nearby source of the contaminants. In the St. Clair River, inputs of the lower Kow compounds, such as QCB and HCB, are from nearby point sources, and therefore levels in the sediment have not yet equilibrated with the water column. In the Detroit River, however, there is evidence of the expected pattern of low Kow compounds being more homogenously distributed in the food web, and considerable compartmentalization being observed for the higher Kow compounds (see Haffner et al. 1990).

Conclusions

Four species of forage fish: *Labidesthes sicculus*, a surface feeder; *Notropis atherinoides*, a facultative surface feeder; *Notropis hudsonius*, a facultative benthivore; and *Pimephales notatus*, a benthivore; were found to have significantly different levels of high Kow organochlorine contaminants. Body burdens were greatest in *P. notatus* reflecting its greater exposure to contaminated sediments. The interspecific differences observed indicated that habitat partitioning was a major factor regulating contaminant levels in these forage fish species. These interspecific differences in contaminant levels have important implications for fugacity models which would predict that these differences should not exist. Although chemical and physiological parameters may determine which contaminants have the potential to bioaccumulate, it is the regulation of exposure through ecological processes that will determine the degree to which that potential is realized.

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List of Figures

BROOK SILVERSIDE

Labidesthes sicculus (Cope)

SPOTTAIL SHINER

Notropis hudsonius (Clinton)

BLUNTNOSE MINNOW

Pimephales notatus (Rafinesque)

FIGURE 1

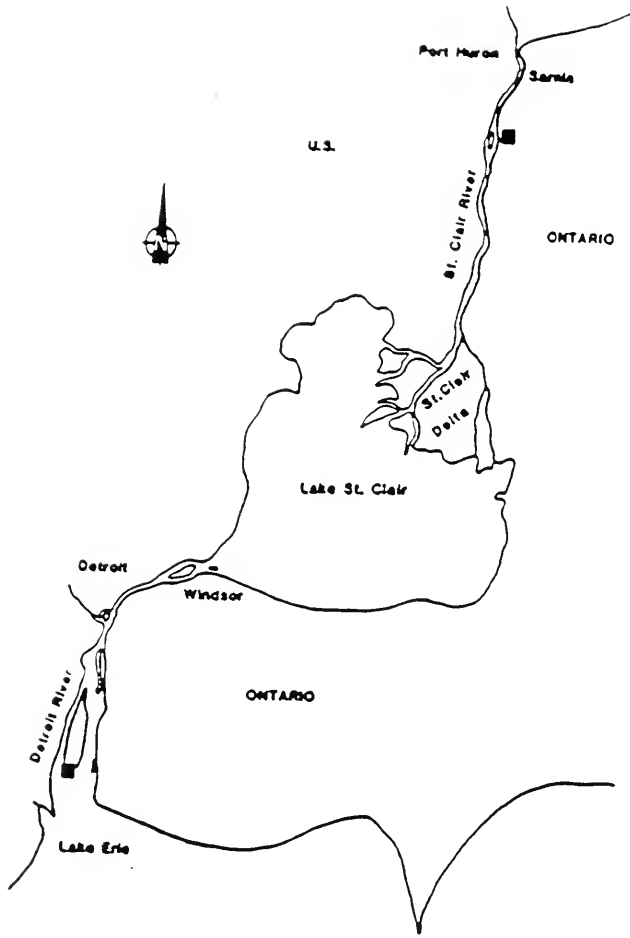
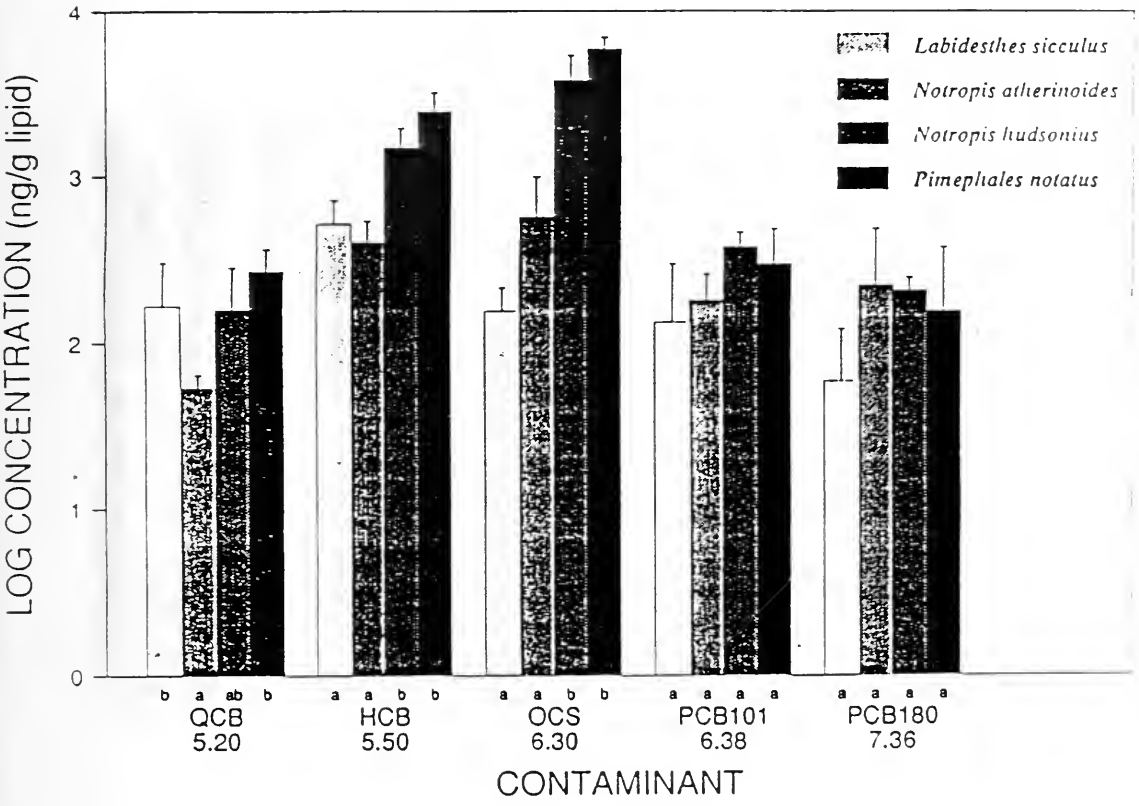

Port Huron
Sarnia
U.S.
St. Clair River
ONTARIO
St. Clair Delta
Lake St. Clair
Detroit
Windsor
ONTARIO
Detroit River
Lake Erie

FIGURE 2


LOG CONCENTRATION (ng/g lipid)
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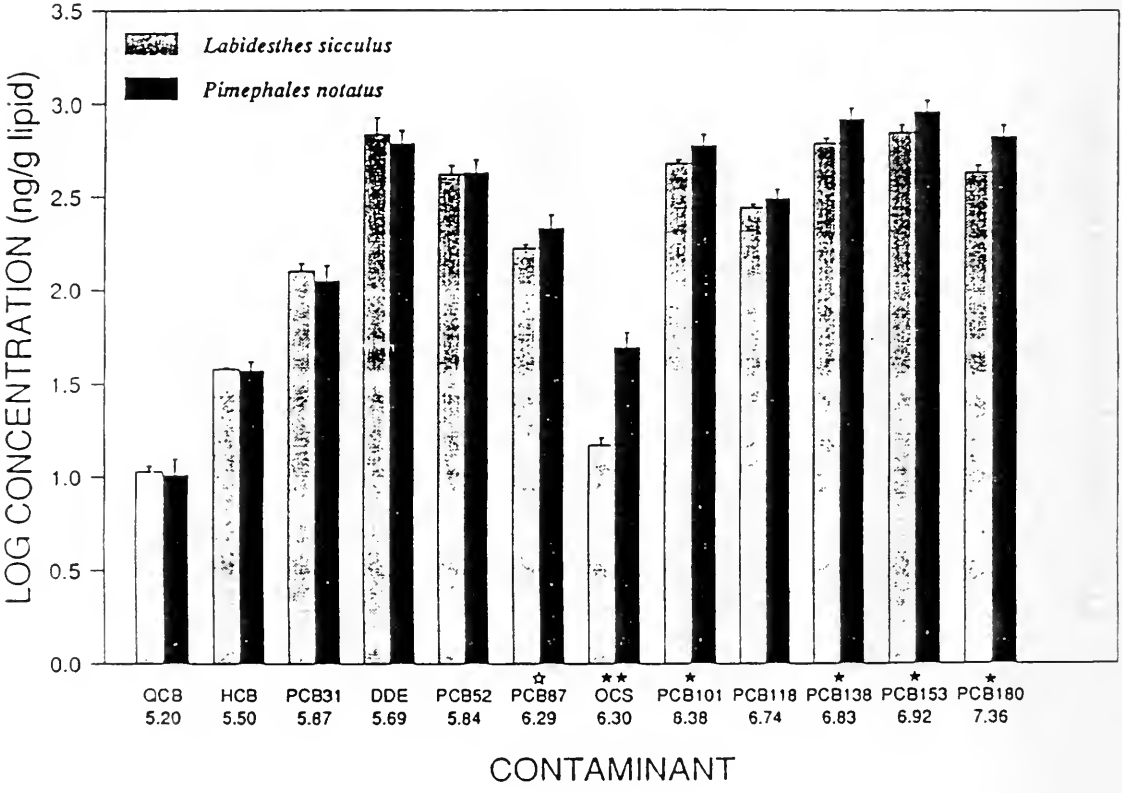
3
2
1
0
Labidesthes sicculus
Notropis atherinoides
Notropis hudsonius
Pimephales notatus
b a ab b
a a b b
a a b b
a a a a
a a a a
QCB
5.20
HCB
5.50
OCS
6.30
PCB101
6.38
PCB180
7.36
CONTAMINANT

FIGURE 3


Labidesthes sicculus
Pimephales notatus
LOG CONCENTRATION (ng/g lipid)
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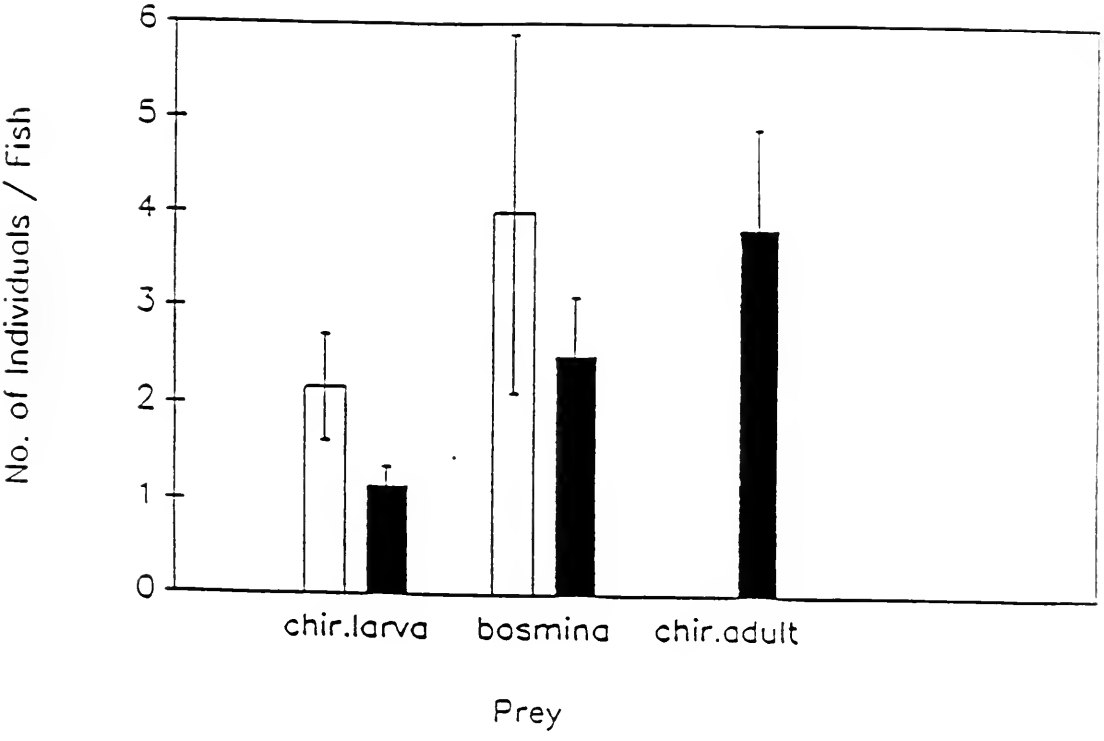
3.0
2.5
2.0
1.5
1.0
0.5
0.0
QCB 5.20
HCB 5.50
PCB31 5.87
DDE 5.69
PCB52 5.84
PCB87 6.29
OCS 6.30
PCB101 6.38
PCB118 6.74
PCB138 6.83
PCB153 6.92
PCB180 7.36
CONTAMINANT

FIGURE 4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5
4
3
2
1
0
chir.larva
bosmina
chir.adult
Prey

FIGURE 5

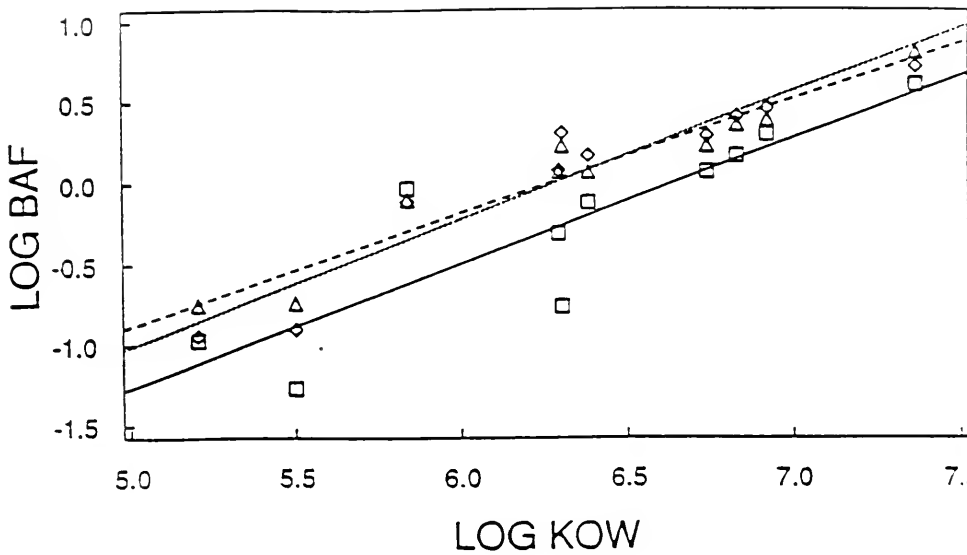

FIGURE 6

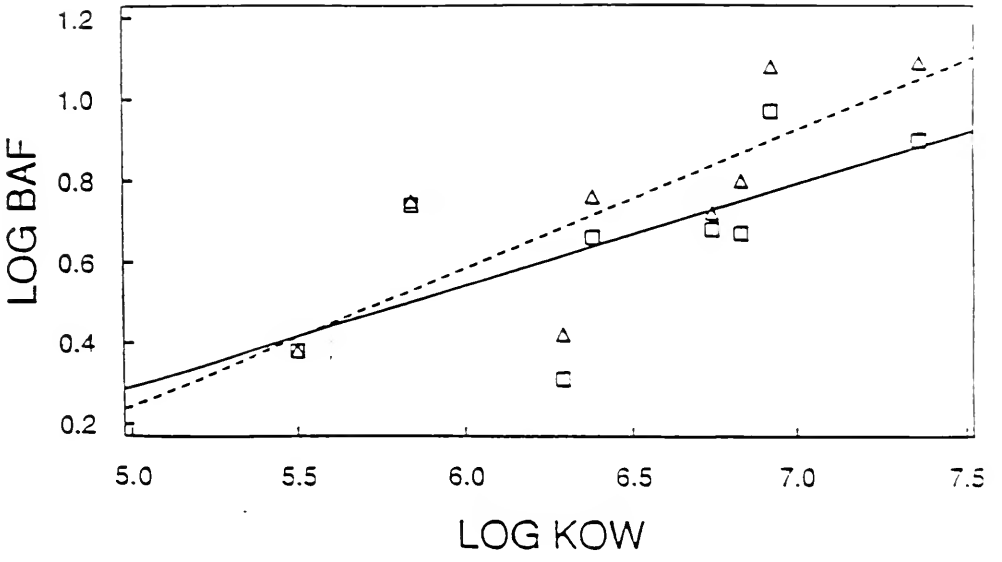

FIGURE 7

FACTORS AFFECTING THE BODY BURDEN OF ORGANIC CONTAMINANTS IN FRESHWATER MUSSELS FROM LAKE ST. CLAIR, ONTARIO, CANADA

by

Bernard Walter Muncaster

A thesis
submitted to the
Faculty of Graduate Studies and Research
through the Department of Biology
in Partial Fulfillment of the requirements
for the Degree of Master of Science at
the University of Windsor

Windsor, Ontario, Canada

1987

ABSTRACT

Biological and physical factors affecting the body burdens of the organic contaminants hexachlorobenzene (HCB), octachlorostyrene (OCS), and four congeners of polychlorinated biphenyls (PCBs) in freshwater mussels from Lake St. Clair, Ontario were investigated. For all contaminants there were no significant differences between the sexes or, with the exception of PCB congener 118, among five indigenous species. Weak negative correlations between body burden and body length existed for all compounds, though the relationship was not significant for OCS and PCB congener 180.

Specimens of Lampsilis radiata (Barnes) and the non-native Elliptio complanata (Lightfoot) were deployed for forty days at four Lake St. Clair sites both to investigate whether the water or the sediment phase represented the primary source of contaminants and the effect of enclosure of the mussels on contaminant uptake. No significant differences in body burdens were detected for any of the compounds between mussels placed in corrals containing purified sand and those with natural sediment, suggesting the water phase represents the xenobiotic source. Among three enclosure types of varying restriction (wire-mesh cages, corrals, and leashes) there were no significant differences in the body burden of each compound. Thus the effect of confinement on uptake is minimal.

Lampsilis radiata individuals from a contaminated region of Lake St. Clair were transferred to an uncontaminated site to

study the xenobiotic purging patterns. The similar ambient levels of PCBs in Lake St. Clair and at the purging site resulted in variable PCB depuration patterns and suggests that the St. Clair River does not represent a major source of PCBs. Hexachlorobenzene displayed the greatest loss of body burden. Sixty-eight percent of the burden was purged during the first four days and only 10 percent of initial levels remained after 59 days. The body burden of OCS decreased less rapidly. Final levels represented 42 percent of the initial burden. The greater affinity of OCS for body tissues may be responsible for the slower depuration. Nevertheless, a change in the ambient concentration of most organic contaminants should be reflected in a detectable alteration in the body burden of these compounds in freshwater mussels.

MODELLING OF FATE AND TRANSPORT OF TOXIC CHEMICALS IN NATURAL WATER WAYS

by

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A thesis
submitted to the
Faculty of Graduate Studies and Research
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for the Degree of Master of Science at
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WINDSOR, ONTARIO, CANADA

1988

ABSTRACT

The response of the St. Clair River and a simple aquatic food chain in Lake St. Clair to the release of hexachlorobenzene (HCB) and octachlorostyrene (OCS), from the the highly industrialized "chemical valley" of Sarnia, is predicted in this study. Two components of the WASTOX fate and transport model are implemented in this project. The time - variable exposure concentration model simulates the transport, transfer and reaction of toxic chemicals in water bodies. It introduces the "moving sediment" layer and calculates the time - variable concentrations for coarse, medium and fine sediment. The food chain component of WASTOX estimates the environmental impact of predicted concentrations in a five - level food chain consisting of phytoplankton, benthic invertebrates, zooplankton, small and sport fish. Phytoplankton and benthic invertebrates are assumed to be steady - state species; time variable concentrations are calculated for all other trophic levels. Two possibilities are considered for the top predator, (a) the sport fish is treated as stationary species in the lake and (b) the sport fish is treated as a migratory species through the St. Clair River Lake system. Two WASTOX submodels are calibrated with 1985 field measurements. The exposure concentration model

indicates that the plume of contaminated water from the multiple sources in the Detroit River travels along the Canadian shoreline and empties into Lake St. Clair via the Channel Ecarte and the South Channel. For both contaminants, about 10 % of total loading is lost in the River due to various processes effecting the toxic pollutant. The food chain component of WASTOX predicts a too rapid response in fish concentration adjustment, caused by the change in the water exposure level. Food chain bioaccumulation of a toxicant due to consumption of contaminated food is greater for OCS than HCB in Lake St. Clair.

FACTORS REGULATING ORGANOCHLORINE CONTAMINANT LEVELS IN FORAGE FISH FROM THE ST. CLAIR AND DETROIT RIVERS

by

Craig Edwards Hebert

A Thesis
submitted to the
Faculty of Graduate Studies and Research
through the Department of Biological Sciences
in Partial Fulfillment of the requirements
for the Degree of Master of Science at
the University of Windsor

Windsor, Ontario, Canada

1990

Abstract

This study examined the factors which regulate levels of hydrophobic organic contaminants in forage fish. Whole body levels of pentachlorobenzene (QCB), hexachlorobenzene (HCB) and octachlorostyrene (OCS) in two species of forage fish: Notropis hudsonius and Pimephales notatus similarly reflected the spatial bioavailability of these compounds in the St. Clair River during 1987. A third species, Labidesthes sicculus, did not show spatial changes in contaminant exposure as consistently. These spatial patterns were contaminant specific reflecting the hydrophobicity of the compound. Contaminant levels in P. notatus most accurately reflected changes in sediment contamination.

Interspecific differences in lipid normalized contaminant levels were examined in fish from the St. Clair and Detroit Rivers. Four species of forage fish: L. sicculus, a surface feeder; N. atherinoides, a facultative surface feeder; N. hudsonius, a facultative benthivore; and P. notatus, a benthivore were collected during 1987-88 from the two sites. Whole body levels of QCB, HCB, OCS, DDE and PCB congeners #31, #52, #87, #101, #118, #138, #153, #180 were determined. Significant interspecific differences in contaminant concentrations were observed for compounds with a log octanol-water partition coefficient greater than 6.0. Highest mean contaminant levels

were seen in P. notatus and lowest levels were observed in L. sicculus. Food and habitat utilization were important factors regulating body burdens in these fish species.

Differences in metabolism may also play a role in regulating interspecific differences in contaminant levels therefore elimination rate constants were determined for P. notatus and L. sicculus. Significant interspecific differences in elimination rate constants for QCB and OCS were observed. However, no difference was observed in the rate at which HCB was eliminated.

FACTORS INFLUENCING THE BIOACCUMULATION OF CHLORINATED HYDROCARBONS BY *HEXAGENIA* NYMPHS (EPHEMEROPTERA : EPHEMERIDAE)

by
Donna Christine Bedard

A Thesis
submitted to the
Faculty of Graduate Studies and Research
through the Department of Biological Sciences
in Partial Fulfillment of the Requirements
for the Degree of Master of Science at
the University of Windsor

Windsor, Ontario, Canada

1990

ABSTRACT

The toxicokinetics of ^{14}C-Hexachlorobenzene by the burrowing mayfly, Hexagenia (Ephemeroptera: Ephemeridae) were studied in laboratory microcosms. First-order uptake and depuration rate constants were measured for water and sediment exposures using a compartmental model. Uptake rate constants from water (kw) averaged 81.0 mL g^{-1} h^{-1}, uptake rate constants from sediment (ks) averaged 0.110 g g^{-1} h^{-1}, depuration rate constant to water (kdw) averaged 0.026 h^{-1} and to water and sediment (kds) was 0.045 h^{-1}. Mayflies exhibited a rapid uptake and elimination of HCB for water and sediment tests with a resultant half-life of 14 hours. Hexagenia nymphs bioconcentrated ^{14}C-HCB from water to an average value of 1,770. Kinetically-derived bioaccumulation factors ranged from 1.80 to 3.06 for sediments with organic matter content of 4.3% to 6.9%. The overall model was used to assess the relative importance of water and sediment as sources of chemical accumulation. Mayflies obtained 98% of the chemical from sediment and 2% from water.

Field-collected mayflies and associated sediments were sampled from a location in Lake St. Clair from July to September, 1987. The samples were analyzed for contaminant concentrations of penta- and hexachlorobenzene, octachlorostyrene and 6 polychlorinated biphenyl congeners. Bioaccumulation factors were calculated as the ratio of chemical concentration in the mayfly on a lipid basis and sediment corrected for the fraction of organic matter. A

linear positive relationship was found between log BAF and the logarithm of the chemicals octanol-water partition coefficient. The BAFs ranged from 0.19 for pentachlorobenzene (log K_{ow} = 5.2) to 1.01 for PCB #87 (log K_{ow} = 7.0). The majority of the chemical BAFs' fell near the chemical equilibrium ratio of 1.0. An analysis of 7 different types of Lake St. Clair sediments revealed a strong linear intercorrelation among particle size, percent organic matter and concentration of organic contaminants.

Behavioural studies examined the relationship between habitat quality and mayfly distribution. A laboratory substrate selection experiment indicated Hexagenia nymphs avoided a coarse-textured sediment but did not discriminated among the 3 finer-grained sediments of varying sand content with equivalent organic composition. In a separate experiment, mayflies actively avoided contaminated sediment in favor of a cleaner substrate of similar particle composition. Natural populations of mayfly nymphs in Lake St. Clair reach maximum abundance in fine, organic-rich sediments and are tolerant of "in situ" chemical concentrations in Lake St. Clair.